Practical PLANT ANATOMY

A. S. FOSTER

D. VAN NOSTRAND COMPANY, INC.

Practical Plant Anatomy

By

ADRIANCE S. FOSTER

Associate Professor of Botany
University of California

NEW YORK
D. VAN NOSTRAND COMPANY, INC.
250 FOURTH AVENUE
1942

PRINTED IN U.S.A.

Press of
GEORGE S. FERGUSON CO.
Philadelphia, Pa.

To my wife

PREFACE

Since a realistic foundation in plant anatomy depends upon thorough laboratory practice, there appears to be a definite need for a guide which will both direct as well as orient the student in his individual studies. The present book has been written from this standpoint and is therefore intended for use in the laboratory. Each exercise contains an introductory section in which an effort is made to summarize briefly but clearly the present status of knowledge of the subject for study. This "Introduction" is in no sense to be regarded as a substitute for collateral reading in the standard texts in plant anatomy and in the selected modern literature which are appended at the end of each exercise. But the author's experience has led to the conviction that a wholly unnatural and artificial gap may easily occur between "theory" and "practice" in the teaching of plant anatomy. To quote from De Bary's classic of 1884, "On the anatomy of plants such an indescribable amount has been written that, in a comprehensive treatise, one or many authors might be cited in reference to every word." The truth of this statement is of course self-evident today and the beginner in anatomy is often confused as well as discouraged by the wealth of detail and maze of controversy presented in many anatomical texts. In the present book, therefore, the aim has been to articulate as far as possible the practical study of laboratory material with the best of modern interpretation and theory. By this means the student, through his own work in the laboratory should be able gradually to acquire a practical basis for the critical evaluation of theory.

The material suggested for study under each exercise has been selected, as far as possible, from types of plants readily available to most teachers. An effort has been made to avoid rare or unusual plants and frequent reference is made to forms of economic importance to man. Wherever it seemed desirable, alternative material has been listed.

In view of the existence of several excellent texts in plant microtechnique, special methods for the preparation of macerated tissue and permanent mounts, as well as the use of microchemical reagents, receive only brief attention in this book. However, a few notes on these topics which may prove valuable to both the teacher and student in the use of this book are included under the "Appendix."

Since teaching methods vary, especially with respect to the nature of the record which the student is required to make of his laboratory work, each exercise contains a list of suggested drawings and special topical reports. This, it is hoped, will permit of selection on the part of the teacher in accordance with the time and emphasis placed on a given topic.

Whatever practical merits the present volume may possess are due to a large degree to the constructive criticisms of numerous students who used the book in its previous planographed form. The exercise on sieve-tube elements has been read and criticized by Dr. Katherine Esau and Dr. A. S. Crafts for whose assistance the author expresses his thanks. I am also grateful for the many helpful suggestions made by Dr. Ernest Ball who served as my laboratory assistant for the past three years. For all errors in fact or interpretation, however, the writer assumes full responsibility.

A. S. F.

Berkeley, Calif.
Oct., 1941

Special Acknowledgments

Quotations from various texts are acknowledged as to pagination and author at appropriate points in this book. For special permission to reproduce these quotations, the author expresses his thanks to the following: Professor T. E. Rawlins and John Wiley and Sons, for the quotation from Rawlins' *Phytopathological and Botanical Research Methods;* University of Chicago Press, for the quotation from Jeffrey's *The Anatomy of Woody Plants;* McGraw-Hill Book Company, for the quotations from Sharp's *Introduction to Cytology* and Eames and McDaniel's *Introduction to Plant Anatomy;* Longman's Green and Company, for the quotations from Priestley and Scott's *Introduction to Botany;* The MacMillan Company, for the quotations from Haberlandt's *Physiological Plant Anatomy*, Strasburger's *Textbook of Botany* and Hayward's *The Structure of Economic Plants.*

TABLE OF CONTENTS

General References

The books listed below constitute the most important general references in plant anatomy and are cited by the author, wherever useful, in the specific reference lists at the end of each exercise. Additional references including recent papers and comprehensive review articles will be found at the end of most of the exercises.

Bower, F. O., Size and Form in Plants. London, Macmillan and Company, Ltd., 1930.

Chamberlain, C. J., Gymnosperms, Structure and Evolution. Chicago, University of Chicago Press, 1935.

———, ——, Methods in Plant Histology. Chicago, University of Chicago Press, 1932.

Committee on Nomenclature, International Association of Wood Anatomists, Glossary of Terms Used in Describing Woods. Tropical Woods 36:1-12. 1933.

De Bary, A., Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns. Oxford, Clarendon Press, 1884.

Eames, A. J., Morphology of Vascular Plants, Lower Groups. New York, McGraw Hill Book Co., 1936.

———, ——, and MacDaniels, L. H., An Introduction to Plant Anatomy. New York, McGraw Hill Book Co., 1925.

Haberlandt, G., Physiological Plant Anatomy. London, Macmillan and Company, Ltd., 1914.

Hayward, H. E., The Structure of Economic Plants. New York, The Macmillan Company, 1938.

Jeffrey, E. C., The Anatomy of Woody Plants. Chicago, University of Chicago Press, 1917.

Johansen, D. A., Plant Microtechnique. New York, McGraw Hill Book Co., 1940.

Mansfield, W., Histology of Medicinal Plants. New York, John Wiley and Sons, Inc., 1916.

Penhallow, D. P., A Manual of the North American Gymnosperms. Boston, Ginn and Co., 1907.

Priestley, J. H., and Scott, L. I., An Introduction to Botany. London, Longmans Green and Co., 1938.

Rawlins, T. E., Phytopathological and Botanical Research Methods. New York, John Wiley and Sons, Inc., 1933.

Sachs, J., Textbook of Botany. Oxford, Clarendon Press, 1882.

Sass, J. E., Elements of Botanical Microtechnique. New York, McGraw Hill Book Co., 1940.

Sharp, L. W., Introduction to Cytology. 3rd Ed. New York, McGraw Hill Book Co., 1934.

Smith, G. M., Cryptogamic Botany, Vol. II. Bryophytes and Pteridophytes. New York, McGraw Hill Book Co., 1938.

Solereder, H., Systematic Anatomy of the Dicotyledons. Oxford, Clarendon Press, 1908.

———, ——, and Meyer, F. J., Systematische Anatomie der Monokotyledonen. Berlin, Gebrüder Borntraeger (apparently as yet incomplete. So far have appeared I Heft, I Teil, 1933; Heft III, 1928; Heft IV, 1929; Heft VI, 1930).

Strasburger, E., Handbook of Practical Botany (trans. by W. Hillhouse). 7th Ed., New York, The Macmillan Company, 1911.

———, ——, Textbook of Botany. 5th Eng. Ed. New York, The Macmillan Company, 1921.

Exercise I

THE PROTOPLAST

I. Introduction.—It is now a well-known fact that protoplasm, which constitutes the physical basis of all life, is organized and "subdivided" in the higher plants into small microscopic units which are termed *cells*. Usually each living cell, regardless of its position, form or function, consists of a wall which encloses a uninucleate protoplast. Exceptions to this typical condition are furnished by the multinucleate protoplasts of certain fibers, vessels and lactiferous elements. Such coenocytes are of considerable theoretical interest with reference to the problem of the origin and significance of the multicellular plant. The so-called "Cell Theory," propounded over a century ago, regards the organism both ontogenetically and phylogenetically as a "cell republic" which has arisen by "the aggregation of a vast number of elementary individuals" or cells (cf. Sharp 1934, pp. 20-24). In contrast, the "Organismal Theory" attaches less importance to the septate condition and regards cellular structure as the *result* of the growth of the organism as a whole. The definitiveness of the cell wall in the tissues of all higher plants doubtless has encouraged the continued wide acceptance of the "Cell Theory" as the more useful concept, at least in any analytical study of plant anatomy.

The term "cell" was originally applied in 1665 by Robert Hooke to each of the numerous "cavities" observed by him in such material as charcoal and cork. Later, with the discovery of protoplasm, the major emphasis was placed upon the living protoplasmic body and the cell wall was regarded as a "lifeless" secretion of the protoplast. At the present time, however, it seems necessary and justifiable to include *both the protoplast as well as its wall under the general term of "cell."* Evidence in support of this viewpoint is furnished (1) by the apparently

intimate relationship existing between the cell wall and the protoplasm during cell differentiation, (2) by the very common occurrence of protoplasmic connections or *plasmodesmata* which penetrate the wall at various points, and (3) by the peculiar behavior of the wall in certain algae (cf. Anderson 1935, pp. 71-72). Many important cell types in vascular plants such as fibers, tracheids, vessel elements, and sclereides consist only of the wall at maturity. However, it is entirely appropriate to designate them as "cells" since the loss of their protoplasts occurs during the later stages of differentiation.

II. The Cells of Plant Hairs.—The cells of many plant hairs furnish very useful material for a study of the protoplast. Because such cells are usually highly vacuolated and hence semi-transparent, they may be easily studied without special preparation or staining. Indeed, in the staminal hairs of *Tradescantia*, the brilliantly colored cell sap in the vacuome provides a splendid optical contrast for the gray cytoplasm and nucleus.

Obtain a transverse section near the tip of the stem of petunia or squash, mount it carefully in water and examine the preparation under *low magnification*. A number of semi-transparent hairs, variable in size and in the form of their terminal cells, will be seen radiating from the edge of the section. Thin sections will show the mode of attachment of the base or "foot" of the hair to the epidermis of the stem. Selecting an uninjured and straight hair, examine its component cells under *high magnification*. Frequently the lower or basal cells of the hair will prove most suitable for this study. By carefully regulating the light and constantly using the fine adjustment on the microscope, the *nucleus*, with its *nucleolus* will be visible. In color, the nucleus will appear gray and slightly opaque. Often the nucleus may appear to be imbedded in the thin layer of *cytoplasm* lining the wall of the cell. Commonly, however, the nucleus is suspended in various positions by a delicate and complex network of *cytoplasmic strands* which extend from the peripheral cytoplasm through the clear watery *cell-sap* of the prominent *vacuome* of the cell. In many of the cells of the hair, small *plastids* may be seen in the peripheral cytoplasm and sometimes in the larger cytoplasmic strands. Well-mounted uninjured sections which have

not been allowed to dry out are suitable for a study of the streaming movement of the cytoplasm as well as changes in the relative position of the nucleus in the hair-cells.

III. Plastids.—Specialized cytoplasmic bodies known as plastids are commonly found in many types of plant cells. Recent studies (cf. Weier 1938) emphasize the many unsolved problems concerning the origin, fundamental structure and activity of plastids, in particular of the photosynthetic *chloroplast*.

Obtain a leaf from the outer region of the terminal bud of *Elodea* and mount it carefully in a drop of water. Examine this leaf first under low magnification, noting that the thin lateral flaps at either side of the "midrib" are composed of only two layers of essentially similar cells. In short, there is no mesophyll as distinct from an epidermis. Study a number of cells in this leaf at varying depths of focus under *high magnification*. Note that all cells contain numerous small discoid *chloroplasts* which typically are in a peripheral position. Many cells, at least in healthy leaves, will exhibit cytoplasmic streaming. Careful examination will show that while the chloroplasts are non-motile in themselves, they are passively carried in a clockwise or counter-clockwise direction by the circulating cytoplasm. Often the chloroplasts are so numerous in a cell that they mask the nucleus. The latter can be more easily observed in the more transparent teeth-like cells which occur irregularly at the margins of the leaf.

Another extremely common type of plastid is the *chromoplast* which frequently produces the red, orange, or yellow color of petals, fruits and certain roots. (*Note:* Since similar colors may be produced by *anthocyanin pigments* in the vacuome, the basis for color in each instance can only be determined by first hand investigation.) The role of chromoplasts in general is obscure and demands further study.

Prepare water mounts of tomato fruit, carrot root, asparagus and rose "hips," and examine them under low magnification. Note the wide variation in size and shape of the chromoplasts. According to Eames and MacDaniels (1925, p. 13) the angular forms of the chromoplasts in the carrot (*Daucus*) "are largely due to the presence of crystals of coloring matter."

IV. Ergastic Substances.—All living cells in plants contain variable amounts of "lifeless" materials which may be collectively designated as *ergastic substances*. They include storage products, waste material, or by-products of protoplasmic activity and in elementary botanical texts are often termed "inclusions." One of the most common examples of ergastic substances is the plant vacuome which consists of a dilute aqueous solution of a wide range of inorganic as well as organic materials.

In addition to the vacuome, many types of plant cells contain further ergastic material in the form of *reserve food* such as starch, proteins and fats or oils. Perhaps the most common type of non-transitory food is starch, which occurs as grains, the size and form of which are highly specific. Obtain a thin, transverse section of the stem of *Pellionia* and after mounting it in water, examine the large parenchyma cells of the cortex under *low and high magnification*. Most of these cells contain starch grains which have developed within the chloroplasts. Often, "fragments" of the chloroplast may be seen at the broad end of the pear-shaped starch grains. The addition of dilute iodine to the section will give the blue color reaction typical for starch. In the tissue of such storage organs as tubers, fleshy roots and cotyledons, starch grains are formed by the activity of *amyloplasts*. These starch-forming plastids lack chlorophyll and are to be regarded as a specialized type of *leucoplast*. Secure a small amount of fresh potato tissue and after gently teasing it with dissecting needles, mount it in water, add dilute iodine and examine under *low magnification*. Note the numerous obovoid starch grains in every cell. Examine a single starch grain under *high magnification* and observe, near the smaller end of the grain, the minute refractive point which is termed the *hilum*. Careful regulation of the light and patient use of the fine adjustment will usually reveal a number of more or less distinct *eccentric layers* arranged about the hilum. For comparative purposes, examine the storage parenchyma cells in the bean cotyledon, noting the difference in the form and position of the hilum in the ovoid starch grains.

A very common type of ergastic substance in many kinds of plant cells is calcium oxalate which appears usually in the form

of conspicuous well-defined *crystals*. It is generally held that such crystals represent an excretory product of the protoplast, being formed by the union of calcium with oxalic acid. The recent monograph by Netolitzky (1929), however, reveals that substances other than oxalic acid may combine with calcium to produce crystalline bodies. Comparatively little is known concerning the factors, chemical and biological, which regulate the rate and mode of crystallization, and which hence determine the form of the adult crystal. Netolitzky (1929, p. 47) concludes that in the final analysis it is "the nucleus which determines which form of crystal will be produced, perhaps by regulating the velocity of crystallization within the cell itself." Examples of the three main forms of plant crystals may now be studied.

1. *Druses* or *sphaerraphides* are compound and consist of more or less spherical aggregates of sharp pointed angular crystals, the whole mass often suggesting in form the mace-head of medieval warfare. Examine the prepared section of the stem of geranium (*Pelargonium*), noting the presence of druses in many of the cortical cells. Transverse sections would suggest that the crystal containing cells are solitary and isolated from one another. But in longisection, it will be seen that frequently the crystal containing cells or *crystal sacs* (cf. Haberlandt) are in short supposed series, each cell of which may contain a druse.

2. *Raphides* are long slender needle-shaped crystals which typically are arranged parallel to one another in definite bundles. Such crystals appear to be most common in the monocotyledons. According to Netolitzky (1929, p. 48) raphides constitute virtually a family characteristic in the Oenotheraceae. Obtain a single living plant of duckweed (*Lemna* sp.) and mount it in water. Note under *high magnification* that many of the transparent cells at the margins of the "thallus lobes" contain prominent bundles of raphides. For comparison, examine under low magnification freshly-cut longi-sections of the stem of *Tradescantia* noting the much larger raphides, many of which may be pulled from the cells during the process of sectioning.

3. *Prismatic crystals* are common in vascular tissue but may also occur singly, or in association with other crystal types in thin-walled cortical parenchyma cells (cf. Exercise VI). Examine prepared slides of the stem of *Tilia* or some similar woody

dicotyledon, noting the solitary crystals in the phloem parenchyma cells.

V. Suggested Drawings and Notes.—

1. Prepare an enlarged drawing of a single hair of either squash or petunia as seen under low magnification showing accurately the number and form of the cells of which it is composed, and its mode of attachment to the stem.

2. Draw a single living cell of a hair as it appears under high magnification. This drawing should portray a "median optical view" and should include the following: cell wall, nucleus (and its visible parts), cytoplasm, vacuole, plastids. *Record*, as laboratory notes, all observations made on cytoplasmic streaming and nuclear "movement" in the material studied.

3. Select a cell from the "midrib" region of the *Elodea* leaf and prepare drawings to show its appearance and contents as seen in surface and median optical views. Describe concisely the variations in the rate and direction of cytoplasmic streaming in cells at different regions of the leaf. What may be the physiological significance of cytoplasmic streaming? Summarize the evidence indicating that plastids do not arise *de novo* in the cytoplasm of plant cells [cf. Sharp (1934, pp. 69-72)].

4. Prepare drawings to illustrate the form and the arrangement of chromoplasts in the material studied.

5. Draw cells from the cortex of *Pellionia*, the potato tuber and the bean cotyledon showing the size and form of the included starch grains.

6. Prepare drawings to show the form and position of the various types of crystals studied.

REFERENCES

1. Anderson, D. B., The Structure of the Walls of the Higher Plants. Bot. Review 1:52-76. 1935.
2. Eames and MacDaniels, Ch. II, p. 6-19.
3. Haberlandt, Ch. X, pp. 530-536.
4. Netolitzky, F., Die Kieselkörper. Die Kalksalze als Zellinhaltskörper. Handbuch. d. Pflanzenanatomie III. Berlin, 1929.
5. ———— . Das trophische Parenchym. C. Speichergewebe. *Ibid.*, IV. Berlin, 1935.
6. Schürhoff, P. N. Die Plastiden. *Ibid.*, I. Berlin, 1924.
7. Sharp, L. W., Introduction to Cytology. 3d. ed. New York, 1934.
8. Weier, E., The Structure of the Chloroplast. Bot. Rev. 4:497-530. 1938.

Exercise II

THE CELL WALL

I. Introduction.—Sperms, eggs and certain other cells of the gametophyte of angiosperms are naked protoplasts and show no evidence of a definite cell wall. Save for these particular exceptions, the zygote and all of the succeeding generations of sporophytic cells which are derived from it are provided from the beginning with a cell wall. Indeed the rigid and often massively-thickened cell wall is frequently cited as a significant distinction between plants and animals; the cells of the latter are either naked protoplasts or else possess thin and less clearly demarcated walls, features which are obviously related to the marked pliancy of many animal tissues.

The form, relative thickness and chemical nature of the cell wall, particularly the secondary wall, provide important and definite characters for separating and classifying cell types in higher plants. Consequently, a preliminary study of wall structure, together with a brief summary of the results of modern research constitute a natural and necessary introduction to the general problem of tissues and cell-types, which will be critically discussed in the following exercise.

II. Plasmodesmata.[1]—The protoplasts of adjacent cells of a wide variety of plant tissues have been shown to be interconnected by delicate threads of cytoplasm, which are termed *plasmodesmata* (for a complete review, cf. Meeuse 1941). Protoplasmic connections seem to support the idea that the cell wall, at least at certain stages in its growth, is an integral part of the living system and not simply a "lifeless" excretion of the protoplast. Knowledge as to the origin and development of plasmodesmata is meagre but there is some evidence that additional or "secondary" connections may arise at different stages in ontogeny.

[1] For special technique to demonstrate plasmodesmata, cf. Crafts (1931).

This problem assumes increased interest with reference to the fate and possible renewed-formation of the plasmodesmata of certain plant cells which are believed to "slide" or push past their neighbors during differentiation (cf. Sharp 1934, pp. 15-18). The function of plasmodesmata is not entirely clear but Haberlandt (pp. 635-638) suggests (1) that they may be essential in transmitting both external and internal stimuli through plant tissues, and (2) that in storage tissue, such as endosperm, they may be "principally concerned with trans-location." Furthermore, considerable evidence has accumulated within recent years which indicates that the plasmodesmata, especially those of sieve-tubes, are the channels through which certain economically-important viruses may travel. Lastly, it is probable that the high degree of correlation between the cells, tissues and organs of the plant may depend upon the presence of plasmodesmata. Livingston, (1935) who maintains that plasmodesmata occur in all the living tissues of the tobacco plant, concludes that "the evidence presented by numerous investigators indicates that actual protoplasmic connections between cells, or plasmodesmata, are generally present throughout all living tissues of higher plants, thus establishing the organism as a definitely correlated entity of interconnecting protoplasts, instead of a community of separate cells."

Endosperm tissue of certain seeds provides useful material for a study of the general features of plasmodesmata. *Obtain* a prepared slide of the endosperm tissue of the persimmon (*Diospyros*) and examine the section under *high magnification*. Note that the greatly thickened walls are traversed by solitary or spindle-shaped groups of very delicate "lines" which are the plasmodesmata. According to Quisumbing (1925), the plasmodesmata are numerous in the walls of the endosperm of *D. discolor* and *D. Ahernii* while in *D. kaki* and *D. ebenaster* "they are few, restricted and grouped at the walls. They occur single or in groups of two, three, four, five or six, and are thicker when single and usually thinner in groups."

III. The Gross Layers of the Cell Wall.—Differentiation and maturation of most plant cells produce marked changes in the area and thickness of the cell wall. Young cells, such as those of embryos and terminal meristems, possess relatively thin walls

which, unless examined with special technique, appear more or less homogeneous in structure. As a cell of this kind enlarges and becomes mature, its wall naturally increases in surface. In addition, the wall may become much thicker, apparently as the result of (1) deposition of new particles of wall substance among those present, a process termed "*intussusception*" by Nägeli, and/or (2) the deposition of successive plates or lamellae of wall material, a process termed "*apposition*" by von Mohl. The process of apposition usually occurs in a centripetal direction with reference to the first or original wall and may result in the almost complete obliteration or occlusion of the cell lumen, as is typical of certain fibers and sclereides (cf. Exercises VIII and IX).

Much confusion exists in textbooks and in the specialized research literature on cell walls with respect to the *subdivision of the wall* into major regions or layers. Distinctions between the successive layers are based upon such criteria as (1) *origin*, (2) appearance when viewed under *polarized light*, and (3) *chemical and physical structure*. Very recently Kerr and Bailey (1934), as a result of an extensive study of material with modern technique as well as a critical survey of the literature, proposed a terminology which has been adopted by Anderson (1935), Hayward and others and which will be followed throughout this book. According to Kerr and Bailey, three main categories of wall layers exist:

1. The *intercellular "layer"* or *substance* which is composed largely of *polyuronides* and is *isotropic* (i.e. dark or non-refringent when viewed under polarized light). Further information on the precise origin of the intercellular layer is urgently needed but provisionally it may be regarded as being derived from the *cell plate* which is produced following mitosis. In tracheary elements, the intercellular layer may be more or less lignified.

2. *The primary wall* which consists largely of cellulose and polyuronides and is *anisotropic* (i.e., bright or refringent when viewed under polarized light). Kerr and Bailey emphasize that the term "primary wall" should be restricted to the original *cambial wall* (in the cells of xylem and phloem) and its homo-

logues in terminal meristems and other thin-walled tissue (e.g. parenchyma). In contrast to the amorphous and "structureless" intercellular substance, the primary wall "is also characterized by possessing plasmodesmata which may be uniformly distributed or aggregated in more or less conspicuous primary pit fields." Primary walls are capable of readjustments and reversible changes in thickness during tissue development. Unless special technique is adopted (e.g. polarized light) the intercellular substance and its adjoining primary walls usually appear as a single non-lamellated partition, especially in tracheary elements. Under such circumstances, the term "*compound middle lamella*" may be applied to this complex of lignified layers.

3. *The secondary wall*, which is often extremely complex, both chemically and physically, and which is normally the most massive of all the main layers of the cell wall. Bailey and Kerr (1935) use the term "secondary wall" to designate "the strongly anisotropic layers of secondary thickening which are formed after a cell has attained its final size and shape." These investigators strongly emphasize that in contrast to the primary or "cambial" wall, the true secondary wall is incapable of undergoing reversible changes in thickness. This is very often the case because of the ultimate disintegration of the protoplast of many cell types which possess definite secondary walls, e.g. tracheids and fibers. According to Bailey and Kerr, most tracheids, fiber-tracheids, and libriform fibers in gymnosperms and angiosperms possess a three-layered type of secondary wall. The inner and outer layers, which are of relatively constant thickness "exhibit strong double refraction and are brilliant" when examined in transverse section in polarized light between crossed nicols. The middle layer, on the contrary, "is dark or noticeably less birefringent" and fluctuates very widely in thickness. Not all secondary walls, however, possess the above type of stratification, exceptions being furnished by certain fibers and by the wall of the cotton hair. From a physical-chemical standpoint, Bailey and Kerr conclude that the central layer of the secondary wall consists of an "extremely complex and firmly coherent matrix of cellulose" within which "lignin" and a wide variety of other organic and inorganic substances may be deposited. The secondary wall of plant

cells is rarely continuous over the entire surface of the adjacent primary wall. In certain tracheary elements of the primary xylem for example, the secondary wall is developed as discrete rings, spirals, bars or as a complex network or mesh, while in other cell types, well-defined thin areas or *pits* occur.

Secure prepared slides of transverse sections of the stem of basswood (*Tilia*) and geranium (*Pelargonium*) and examine the parenchyma tissue of pith and cortex under *high magnification.* Note carefully the thin, apparently unstratified "compound middle lamella" of each cell and the prominent *intercellular air spaces.* The very slightly thickened "walls" of these cells appear to represent the original intercellular substance and the two adjacent primary walls of the terminal meristem cells from which they originated.

Examine under both *low* and *high magnification* the extremely thick-walled *bast fibers* of *Tilia* noting the very thin, continuous compound middle lamella, the thick, obscurely stratified secondary wall and the much reduced lumen of each cell. Occasionally, in both transverse as well as longisections, very small canal-like pits will be visible in the secondary wall.

IV. Pits.—With few exceptions, the secondary wall of plant cells is interrupted by small cavities or recesses which are termed *pits.* These thin areas in the secondary wall vary widely in size, structure and arrangement and, since they exhibit some constancy depending upon the type of cell, they provide significant criteria in comparative studies, especially of xylem cells. Pits typically occur in pairs; i.e., a thin area in the secondary wall of a given cell normally lies opposite a similar recess in the adjacent cell. Hence the term "*pit-pair*" designates the usual condition and is contrasted in meaning with "*blind pit*" which is a pit "without a complement opposite to an intercellular space" (cf. Glossary of Terms Used in Describing Woods, p. 5). *Each* member of a simple pit-pair consists of (1) the *pit cavity,* which is the actual space within the secondary wall, and (2) the *pit aperture* or opening into the cavity. The members of a pit-pair are separated from one another by a common *pit membrane* which represents a discrete portion of the presumably modified intercellular substance and the two primary walls. Compara-

tively little detailed information is available regarding the ontogeny of pits. Possibly their usual paired character is associated with the fact that at least in living cells, the pit membrane is penetrated by plasmodesmata which thus may determine the opposite position of the pits. At any event it is clear that pit-pairs arise on the *primary pit fields* of meristematic cells. These pit fields which are defined (cf. Glossary of Terms Used in Describing Woods, p. 4) as thinner areas of "the intercellular layer and primary walls" are observable in cambial initials as well as in the so-called primordial meristem of the shoot apex of certain seed plants (cf. Foster, 1938, 1939a, in "References" to Exercise III). From the standpoint of function, pits are believed to facilitate the process of diffusion between adjacent cells.

Pit-pairs may be conveniently classified under four major types, viz.:

1. *Simple pit-pairs*, which are typical of cells which retain a protoplast throughout their functional life, are particularly well developed in parenchyma cells. In *face view*, the *aperture* appears as a circular, elliptical or even irregular area. In macerated tissue,[1] simple pit-pairs in this view appear as refractive red points of light. A recognition of this optical characteristic will help to distinguish simple pits from particles of protoplasm or other substances lying free in the cell lumen. In *sectional view*, the *cavity* of each member of the pit-pair is usually of equal diameter throughout and there is no overarching rim or border produced by the adjoining secondary wall.

Obtain a preparation of macerated secondary xylem of the stem of the trumpet-creeper (*Tecoma radicans*) and examine it under *low magnification* noting the numerous wood parenchyma and xylem-ray parenchyma cells. These cells are box-like in form and occur singly or in groups depending upon the extent to which the xylem has been macerated. Study a connected group of parenchyma cells under *high magnification* and investigate the size, structure and position of the simple pit-pairs as seen in surface and sectional views. In all studies of this kind, it is essential to secure critical illumination and to use the fine adjustment of the microscope constantly.

[1] Cf. Appendix, pp. 140-141 for the technique of macerating plant tissue.

2. *Bordered pit-pairs* are typical of dead water-conducting cells, notably tracheids and vessel elements. In contrast to the previous type, the cavity of each member of the pit-pair is over-arched by a rim-like development of the secondary wall which is termed the *border*. As seen in face view, the pit aperture is circular or broadly elliptical. In *median section view*, the border over-arching each pit member is apparent and, an additional structural peculiarity is observable, namely the *torus*. The latter is a discoid, central, thicker portion of the pit membrane which is slightly wider than the diameter of the pit aperture. The remainder of the membrane is much thinner and sufficiently pliable so that under certain conditions the torus may be pressed against one or the other of the two pit apertures. To understand clearly the structure of a bordered pit-pair, it must be visualized in both face and sectional views. Reference to Eames and MacDaniels (1925, pp. 27-30, Figs. 15, 16, 17 and 21) and to Jeffrey (1917, pp. 5-6, Figs. 4 and 5) will prove helpful.

Obtain a preparation of macerated xylem of the stem of *Pinus* and examine it *under low magnification*. Bordered pit-pairs are large and obvious in the tracheids, which are elongated cells with acute or blunt tips. Select a suitable tracheid and study the appearance of the bordered pits in *face view under high magnification*. Observe that each pit appears as three concentric outlines. The outermost circle demarcates the edge of the pit cavity, the intermediate circle represents the edge of the torus and the somewhat refractive innermost circle is the pit aperture. In many tracheids, the successive bordered pits are more or less clearly set apart from each other by "eye-brow" or rim-like ridges, termed *crassulae*. These are interpreted as "thicker portions of the intercellular layer and primary walls between primary pit fields" and in the past have been designated as "Bars of Sanio" and "Rims of Sanio" (cf. Glossary of Terms Used in Describing Woods, p. 4). The structure of bordered pit-pairs in sectional view can be effectively studied in macerated material if the *edge* of the pitted walls are turned towards the observer. In order to study critically the pit membrane and the torus, it is necessary to examine thin, properly-stained radial sections of pine xylem.

3. *Half-bordered pit-pairs* represent "an intercellular pairing of a simple and a bordered pit" (Glossary of Terms Used in Describing Woods, p. 5) and occur when *living* parenchymatous cells develop in contact with *dead* tracheary elements. According to a frequent opinion expressed in texts, the pit-member on the side of the living cell is simple while its mate on the side of the tracheid or vessel is bordered. Frost (1929), however, in a study of the nature of pitting between tracheary and parenchymatous cells in angiosperm xylem, has found that this conception has no general validity. He concludes that "fully bordered, half-bordered and simple pits are characteristic features between tracheary cells and vascular parenchyma" and that "the type of pitting on the wall of the parenchyma cell is controlled largely by the degree of specialization of the vessel or fiber which lies next to it." Obviously the whole question of pitting in plant cells demands further investigation, from both a comparative as well as an ontogenetic point of view.

Secure prepared slides of conifer and dicotyledonous secondary xylem and investigate *under high magnification* the nature of the pitting between wood parenchyma or wood ray cells and the connected tracheary elements.

4. *Vestigial pit-pairs* are typical of thick-walled wood and bast fibers. In these cells, the secondary wall is greatly thickened and the pits are often so reduced in size and number as to appear truly "vestigial" or functionless. The vestigial pit-pairs of typical wood fibers are usually interpreted morphologically as reduced and highly modified bordered pit-pairs. This conclusion is based (1) on the belief that the wood fiber has developed phylogenetically from the tracheid, and (2) on the fact that a closely graded series of intermediate conditions between typical "bordered pit-pairs" and "vestigial pit-pairs" can be seen in comparing the tracheids, fiber-tracheids and fibers in the xylem of the same plant. In typical vestigial pit-pairs of wood fibers, the pit-cavities although circular are relatively small, the border is greatly reduced in size or absent and a torus is frequently lacking. The most distinctive feature of this type of pitting, however, consists in the elongated *slit-like apertures* which instead of being *opposite* (as is true of the circular apertures of the members of

a pair of bordered pits) are crossed. *Furthermore,* each slit-shaped aperture is connected with the pit cavity by a channel having the form of a flattened funnel (cf. Eames and MacDaniels, 1925, p. 33, Figs. 21-22). The vestigial pit-pairs of bast fibers are frequently less complex, and consist of small circular apertures and very narrow tubular cavities. Such pits are often regarded morphologically as specialized simple pits.

Obtain a preparation of macerated secondary xylem of the sycamore (*Platanus*) and examine it under *low magnification,* noting the numerous long, acuminate *wood fibers.* Careful study of these fibers under *high magnification* will reveal the characteristic arrangement of the vestigial pits with their narrow apertures and inconspicuous "halo-like" cavities. Occasional fibers may be turned in such a way that the vestigial pits or pit-pairs may be visible in *sectional view.* A study should also be made of pit structure as revealed in prepared and stained longisections through the xylem.

Make a comparative study of the vestigial pit-pairs of the bast fibers of *Tilia* or *Platanus* with the use of macerated as well as stained and sectioned material.

V. Suggested Drawings and Notes.—

1. Prepare drawings showing the arrangement and approximate number of *plasmodesmata* in a small group of connected endosperm cells of *Diospyros.* Briefly summarize the possible importance of the presence of plasmodesmata with reference to the trans-location of organic materials in plants.

2. Draw a group of pith or cortical parenchyma cells from the stem section of *Tilia* or *Pelargonium* showing and labeling the following: *compound middle lamella, protoplast, intercellular air spaces.* In what kinds of plant tissue are *intercellular air spaces* likely to be most prominent? Explain, from a physiological viewpoint (cf. Haberlandt, Ch. IX).

3. Draw a group of bast fibers of *Tilia* as seen in the transsection of the stem, showing and labeling the following: *compound middle lamella, secondary wall,* and *lumen.* What might be the "cause" of the deeply-stained thickenings frequently visible at the common point of contact between several bast fibers?

4. Draw several connected wood-parenchyma or wood-ray cells from the macerated xylem of *Tecoma radicans*, showing clearly the size, structure and arrangement of the *simple* pit-pairs as seen in both face and sectional view. Label the following: *compound middle lamella, secondary wall, pit aperture, pit cavity* and *pit membrane*.

5. Draw, on a large scale, a single tracheid from macerated pine xylem, showing the form and arrangement of *all* pits (bordered and simple) as seen in face view. Label carefully. Prepare drawings based on the study of the longisections of pine wood showing *sectional views of bordered pit-pairs* as well as the type of pitting between tracheids and wood rays. In these drawings label the following: *compound middle lamella, secondary wall, border of pit, aperture of pit, cavity of pit, pit membrane, torus* and *crassulae*.

6. Prepare drawings, based upon the study of macerated and sectioned wood and bast fibers of *Platanus* and *Tilia*, showing the arrangement and structure of *vestigial pit-pairs* in both face and sectional views.

REFERENCES

1. Anderson, D. B., The Structure of the Walls of the Higher Plants. Bot. Rev. 1:52-76. 1935.
2. Bailey, I. W., and Kerr, T., The Visible Structure of the Secondary Wall and Its Significance in Physical and Chemical Investigations of Tracheary Cells and Fibers. Jour. Arnold. Arb. 16:273-300. 1935.
3. Bonner, J., The Chemistry and Physiology of the Pectins. Bot. Rev. 2:475-497. 1936.
4. Crafts, A. S. A Technic for demonstrating plasmodesmata. Stain Technology 4:127-129. 1931.
5. Eames and Mac Daniels, Ch. II, pp. 19-38.
6. Frost, F. H., Histology of the Wood of Angiosperms, I. The nature of the pitting between tracheary and parenchymatous elements. Bull. Torrey Bot. Club. 56:259-264. 1929.
7. Haberlandt, Ch. I, pp. 43-50; Ch. XIII, pp. 635-638.
8. Hayward, Ch. I, pp. 5-11.
9. Jeffrey, Ch. I.

10. Kerr, T., and Bailey, I. W., The Cambium and Its Derivative Tissues, X. Structure, optical properties and chemical composition of the so-called middle lamella. Jour. Arnold Arb. 15:327-349. 1934.
11. Livingston, L. G., The Nature and Distribution of Plasmodesmata in the Tobacco Plant. Amer. Jour. Bot. 22:75-87. 1935.
12. Meeuse, A. D. J., Plasmodesmata. Bot. Rev. 7:249-262. 1941.
13. Quisumbing, E., Continuity of Protoplasm in Endosperm Cells of *Diospyros*. Bot. Gaz. 80:439-449. 1925.
14. Sharp, L. W., Introduction to Cytology. 3rd ed. New York. 1934.
15. van Wisselingh, C., Die Zellmembran. Handbuch d. Pflanzenanatomie, III. Berlin, 1924.

Exercise III

MERISTEMS

I. Introduction.—A *meristem* may be defined as a specific region in the plant body where cells are engaged chiefly in *division* and *enlargement*. Meristems thus represent *embryonic areas* and can be conveniently classified according to *position* as *apical meristems* and *lateral meristems*. The former type is illustrated by the apex or "growing point" of the root and the shoot, the latter by the vascular and cork cambia. In addition, many authors recognize "intercalary meristems" which are said to occur *between* areas of permanent or mature tissue, as for example at the base of the leaf in certain monocotyledons. A more critical study of the so-called "intercalary meristems" is urgently needed, however, and attention will be again directed to this problem in the exercise dealing with the leaf (Exercise XIII).

The maintenance of meristems at certain restricted regions of root and shoot is responsible for the distinctive "open system" of growth characteristic of all higher plants. This consists in the *continuous formation* of *new tissues* and *new organs* throughout the life of the individual. The magnitude of this type of "unlimited" ontogeny is most impressively shown in long-lived woody perennials in which each season's growth is accomplished by the formation of *new* shoots, reproductive structures, and roots, as well as by an increase in diameter of the older roots and stems. When the open system of growth is further examined, it becomes clear that there are varying degrees of "permanence" in the various meristems of a plant. Thus the apical meristems of the shoot and root in many perennials appear capable of indefinite life and activity. Actually, of course, various factors such as malnutrition, insufficient water, injury, etc., may result in the death of the shoot or root apex. Furthermore, the phenomenon of *correlation*, in this case involving the *relative growth* of main stem or main root as compared with its laterals, becomes a com-

plicating factor. Another example of a theoretically "indefinite" or "permanent" meristem is furnished by the *vascular cambium*, which may continue to produce annual increments of phloem and xylem for hundreds or, in the genus *Sequoia*, thousands, of years. Obviously, the maintenance of an indeterminate type of meristem, such as a shoot apex or the cambium, requires that there shall be a continuous new formation or "regeneration" of the meristem as development takes place. In other words, a certain restricted portion of such meristems remains *indefinitely* in the embryonic state and does not pass into the zone of maturation. In contrast, the meristems of determinate organs, such as leaves and fruits, function for only a comparatively restricted period, and eventually all embryonic tissue passes into a state of maturity. Evidently these differences in the functional life of meristems are of fundamental morphological importance, but the nature of the factors, genetical and physiological, which control them are very poorly understood at present.

From an histological standpoint, a meristem is a "tissue" composed of "undifferentiated" or meristematic cells. According to the classical viewpoint, which is still retained in many textbooks, the tissue composing meristems possesses certain distinctive juvenile characteristics which seem to demarcate it from the various types of functionally-mature "permanent" tissues. Among the "negative" characters usually assigned to meristematic tissue are: (1) the absence of intercellular spaces; (2) the absence of thickened or pitted walls; and (3) the absence of prominent ergastic materials in the cytoplasm. If, however, undue emphasis is placed upon such morphological features, a narrow and rather arbitrary concept of "meristematic tissue" inevitably results. A good example of the restricted concept of meristem is found in Priestley and Scott's recent (1938, p. 208) "An Introduction to Botany." They state: "It is customary to speak indifferently of any dividing tissues of the shoot apex as meristematic, but in view of the fundamental character of the differences in cell behavior, it is proposed in this book to restrict the terms meristem and meristematic to the dense cells which are devoid of obvious water vacuoles and have no intercellular spaces between them, whilst the vacuolating cells will be spoken of as

exhibiting vacuolating cell growth and division." Priestley's effort to demarcate meristems on the basis of the absence or lack of prominence of vacuoles is not supported by comparative studies. For example, the apical cell and its most recent segments in many lower vascular plants are highly vacuolate in character and it is only at some distance from the summit of the apex that small "dense" cells are found to occur (Eames and MacDaniels, 1925, Fig. 28; and Zirkle, 1932, taf III, Fig. 16). Likewise, in the shoot apices of *Ginkgo biloba* and *Cycas revoluta*, *Zamia*, and *Dioon edule* a more or less well-defined *central group* of enlarging vacuolated cells, surrounded by smaller and more densely-cytoplasmic cells is present (*cf.* Foster, 1938, 1939a, 1940, 1941b). These examples clearly show that the relative *position* and *extent* of "zones" characterized by the predominance of cell division or cell enlargement are variable in the shoot apex of vascular plants (Foster, 1941a; Boke, 1941). In short, cell division and conspicuous vacuolation are not processes confined in Priestley's sense respectively to the summit and lower portion of a shoot apex. On the contrary, these processes may overlap at the same *level* in a growing apex. Doubtless the most significant evidence of the vacuolated character of meristems has been secured by recent studies on living tissue. The work of Bailey (1930) and Zirkle (1932) on the vascular cambium and primary meristems respectively has indicated that *all meristems are vacuolated*, and furthermore, that the form of the "vacuome" varies within wide limits at different seasons of the year and at different stages of growth in the same type of meristem.

From the preceding brief critique it should be evident that it is impossible in the light of present knowledge to frame an adequate "definition" of meristematic tissue. On the contrary, it seems increasingly clear as investigation proceeds, that we have to deal with various and possibly distinct types of "meristem," at least from a physiological viewpoint. How the organization and growth of meristems is related to the orderly *progressive differentiation* of tissues from apical or lateral meristems constitutes one of the most challenging problems in modern botany. Further insight will come when the results of comparative observation are checked by experimental studies. It seems likely that

the complex phenomena of *regeneration* or *regressive differentiation*, when they are better understood, may be expected to shed important light on the fundamental nature of meristems. (Cf. Bloch, 1941; Sinnott and Bloch, 1941a, 1941b.)

In the present exercise, a preliminary study will be made of the apical meristems of root and shoot, and of the vascular cambium. Further experience with these meristems as well as with the cork cambium will be gained particularly in the exercises devoted to the anatomy of root, stem, and leaf.

II. Apical Meristems.—

1. *The shoot apex.* The classical investigations of C. F. Wolff (1759) on bud development showed that new leaves and new stem tissues are traceable in origin to the delicate tip of the shoot. Wolff designated this region as the "punctum vegetationis," a term which has been rather freely translated as the "growing point." Despite the widespread adoption by anatomists of the expression "growing point," this term carries an inaccurate implication and in the present book will be replaced by the more appropriate and non-committal designation of "shoot apex." This decision is based upon the fact that the *chief significance* of the so-called growing point is that it represents the *region of initiation* of the primary organization of the shoot, rather than a localized area or "point" of "growth." As a matter of fact, if "growth" is regarded as an increase in size of cells, tissues, and organs, this process is obviously at a minimum in the "growing point."

Great variation obtains with respect to the *form* and *dimensions* of the shoot apex of seed plants. As seen in median longisection view, the apex commonly has the form of a mound or low dome. In *Elodea*, *Myriophyllum*, and *Hippuris*, however, the shape of the shoot apex is that of a slender, blunt-tipped cone (cf. Louis, 1935, pp. 126-130 and Pl. IX, Figs. 77-78). The apex of dicotyledons with decussate phyllotaxis (e.g., *Syringa*, *Lonicera*, *Ligustrum*, etc.) is particularly suitable for developmental studies because the initiation of each pair of foliar structures is preceded by a notable and symmetrical *expansion* of the terminal meristem. Since this process is repeated each time a pair of leaves is produced, the apex exhibits a rhythmical alternation of

what Schmidt (1924) called "minimal" and "maximal" areas (cf. also Louis, 1935, Pl. II, Figs. 20-21; and Cross, 1937, Figs. 10-11). This situation emphasizes the fact that the form and the dimensions of the shoot apex are likely to vary depending upon whether an active or dormant apex is measured as well as upon the particular phase in shoot development which is under examination. Extremely few careful measurements have been made of the shoot apex of seed plants, and no generalizations are possible at present. Apparently, however, the angiosperms typically possess rather small apices which range in diameter from 90μ in certain grasses to 500μ in some of the palms. Possibly 130-200μ may prove to represent a frequent range in diameter of the apex in dicotyledons (Boke, 1940). The width of the shoot apex of some conifers, of *Ginkgo biloba*, and of *Zamia* slightly exceeds that of "typical" angiosperms. But in the Sago Palm (*Cycas revoluta* Thumb.), the shoot apex may attain the relatively enormous diameter of 3.5 millimeters, a dimension greatly exceeding that recorded for any vascular plant (cf. Foster, 1940). The nature of the relationship between size and form of the apex, on the one hand, and the morphology and primary anatomy of the shoot, on the other, is obviously complex and awaits further comparative studies for its solution (Bower, 1930, Ch. XII; Foster, 1939b, 1940, 1941a, 1941b).

When a thin median longi-section of the *shoot apex of an angiosperm* is examined under the microscope, two principal zones or regions are usually distinguishable, viz.: (1) the *tunica*, which consists of one or more discrete superficial layers of cells, and (2) the *corpus*, which is a "core" occupying the center of the apex and exhibiting an irregular or "random" arrangement of cells.[1] The differences in cell arrangement in tunica and corpus

[1] In both of these zones, the cells are relatively small and in sectional view appear "isodiametric" in form. Little is known, however, about the shape of such cells when regarded as three-dimensional structures. According to Priestley and Scott (1938 pp. 201-202) macerated cells of the apex "appear as rather irregular, many-sided figures, the facets of which are mainly hexagonal or square." By compressing spheres of plasticine, these investigators obtained 12-sided bodies which they assume are similar in form to meristem cells. They conclude that "the shape of the cells is thus explained as the natural result of the growth and division of plastic bodies under mutual pressure." (For further information on the problem of form in isodiametric plant cells cf. Ex. VI, pp. 57-58.

result from differences in the *direction of growth* and *plane of cell division* in these zones. In the tunica, *surface growth* accompanied by repeated *anticlinal divisions* predominates, resulting in the maintenance at the summit of the apex of a more or less regular and constant series of shell-like layers. On the sides or flanks of the apex, however, the distinctness of the inner tunica layers is somewhat lost, chiefly because of the *periclinal* and *oblique divisions* which appear in them during the *initiation* of *foliar structures* and *lateral buds*. In contrast to the tunica, *growth in volume* is characteristic of the *corpus*, and the sequence in the successive planes of cell division is variable and usually very irregular. Tunica and corpus thus represent *two interdependent zones* in the shoot apex, and their extent and behavior may be expected to fluctuate, depending upon the systematic position of the plant in question as well as upon the phase of development of the plant itself. Modern studies have shown that the number of tunic layers varies from one in grasses and *Scrophularia nodosa* to as many as five or six in *Hippuris*. Unfortunately, no detailed survey along broad systematic lines has yet been attempted, so that the phyletic significance, if any, of differences in the number of tunic layers is quite obscure at present. The classical "Histogen Theory" of Hanstein (1868) attempted to assign specific destinies or "prospective values" to the various layers and to the central core of the shoot apex. In contrast, the concept of tunica and corpus, which originated with Schmidt (1924), is non-committal with respect to the *nature of the tissues produced by these two zones*. Recent studies justify Schmidt's cautious viewpoint. In certain angiosperms [e.g., *Viburnum rufidulum*, Cross (1937)], the corpus is exclusively concerned with the production of the pith, while in other plants *Carya Buckleyi* var. *arkansana*, Foster (1935); *Morus alba*, Cross (1936), the provascular tissue and inner region of the cortex, *as well as the pith*, originate from the corpus zone. In *Hippuris* and *Myriophyllum*, the corpus gives rise to the central pith-less stele of the axis (Louis, 1935, pp. 128-130, Pl. IX, Figs. 77-78), simulating in this respect the histogenesis characteristic of many roots. The "prospective significance" of the various layers of the tunica also varies, particularly with respect to their role in the

initiation of leaf and bud primordia (Foster, 1936). While the outermost tunic layer very commonly behaves as a "dermatogen" and produces exclusively the epidermal system of leaf and stem, the apices of *Triticum* and *Avena* furnish interesting exceptions. In these grasses, the foliage leaf originates largely if not exclusively from the single tunic layer which exhibits both periclinal and anticlinal divisions at the early phases of foliar development. Doubtless similar conditions will be discovered in other angiosperms.

A proper study of the form and structure of the shoot apex in seed plants and of the origin of primary stem tissues and leaves is only possible if both *longitudinal* and *transverse* serial sections are available. Since the choice of bud material will depend upon many factors, no detailed description of a specific shoot apex will be made in this book. Instead, suggestions as to the advantages and special features of several available types of apices will be given. With the information presented in the earlier portions of this exercise and in the literature cited, the student should have no difficulty in interpreting the general organization of any angiospermous shoot apex. The mound- or dome-shaped form of apex, with several tunic layers, is well illustrated in such genera as *Carya*, *Morus*, *Rhododendron*, *Acacia*, *Syringa*, *Rosa*, *Sambucus*, and *Helianthus*. Aside from minor variations, the origin of leaves and the differentiation of provascular strands ("pro-cambium") and "rib meristem" are similarly shown in all of these genera. "*Rib meristem*," a concept developed by Schüepp (1926), is a type of primary meristem which in a longi-sectional view of a shoot apex appears as a tissue composed of vertical filamentous groups of vacuolating-dividing cells. This meristem typically differentiates into the parenchyma tissue of cortex and pith. The slender cone-shaped apices of *Elodea* or *Hippuris* are instructive, providing median longi-sections are examined. Apices of these genera are particularly useful in demonstrating the mode of origin of the small leaf primordia from the tunica zone, as well as showing the early demarcation between cortex and the pith-less stele. Preparations of the shoot apices of monocotyledons should also be studied. The apex of *Tradescantia* is of interest since the demarcation between

tunica and corpus zones is not always clear. Furthermore, the relation of rib meristem and provascular areas to the young nodes and internodes is clearly shown in this genus (cf. Rüdiger, 1939, and Ball, 1941). For comparative purposes, a study should also be made of the shoot apices of various gymnosperms. Here the choice of material is often very limited and hence specific recommendations may be of little value. But the apices of vigorous growing shoots of *Picea*, *Abies*, or *Cedrus* are readily sectioned and all agree in the absence of the tunica-corpus type of zonation characteristic of angiosperms. Instead, a small group of initials is situated at the summit, from which arise two major tissue-areas or zones, viz.: (1) an outer *peripheral zone*, which produces the leaves, epidermis, cortex, and provascular tissue, and (2) an inner or *central tissue zone* which produces exclusively the pith. The possible phylogenetic significance of this type of apex is discussed in several recent papers (Foster, 1939b, 1941a; Cross, 1939, 1941). Apices of *Ginkgo* (Foster, 1938) and of some type of cycad (*Zamia*, Johnson, 1939; *Cycas revoluta*, Foster, 1939a, 1940; *Dioon*, Foster, 1941b) are also worthy of the student's time, particularly because of the interesting phylogenetic as well as morphogenetic problems which are raised by their unique growth and structure.

2. *The Root Apex.* The apex of the root differs fundamentally from that of the shoot in the presence of a *root cap*. The latter is a thimble-shaped or conical structure which occupies the true physical apex of the root and which acts as a "buffer" for the delicate meristematic tissue which is thus *subterminal* in position. Great variation exists with respect to the histogenetic relationships between the root cap and the subterminal meristem. Indeed, the differences are sufficiently evident to make necessary the designation of a number of "types" of root apices which are distinguished (1) by the mode of origin of the cap, and (2) the relation of the various so-called "histogens" to the origin of the primary tissue regions in the root proper. (Haberlandt, 1914, pp. 89-94, and Hayward, 1938, pp. 44-48.) It is an interesting fact that while the highly deterministic scheme of Hanstein (1868) has been largely abandoned for the shoot apex, the structure and growth of the root apex is still generally inter-

preted in terms of the "histogen theory" (Hayward, 1938, pp. 44-48; von Guttenberg, 1940). While it is true that the absence of foliar structures in roots makes it relatively easy to determine the point of origin of a given tissue, it may well be questioned whether Hanstein's concepts are any more justified for the root than for the shoot. It seems evident, at any event, that a broad systematic survey of the structure and behavior of the root apex in angiosperms and gymnosperms would remove the problem from the highly *formalized position* which it now occupies.

Secure serial longitudinal and transverse sections of some of the principal "types" of root apices, viz.: (1) the "grass type," characterized by the possession of a discrete meristem termed the *calyptrogen* which exclusively propagates the root cap, and by the origin of "dermatogen" and "periblem" from a common initial group; (2) the "*Allium* type" in which, according to Hayward (1938, p. 46) "the root cap, epidermis, and cortex arise from a common group of initials two cell layers in thickness, and within this zone is a sharply defined plerome;" (3) the "*Helianthus*" type which is believed to represent the most common type in dicotyledons, and in which "the plerome and periblem are sharply defined; and outside the latter is a common initial layer which produces the root cap and the epidermis" (Hayward, 1938, p. 46, and Haberlandt, pp. 89-90, Fig. 19); and (4) the "*Pisum*" type in which a transverse initiation zone is the common point of origin of root cap as well as the primary tissues of the root. In this type, which is found in *Cucurbita* and many Leguminosae, well-defined "histogens" are not recognized (Hayward, p. 47, Fig. 17). Regardless of "type," careful inspection of median longi-sections *under low* and *high magnification* will reveal the successive "zones" of *cell origin*, *cell elongation*, and *cell maturation*. The "*rib meristem*" of the outer peripheral region of the root tip is particularly useful in studying these zones, because of the extremely regular arrangement of the cell rows and the gradual changes in size, shape, wall thickness, and degree of vacuolation of the component cells. Note in contrast the relatively short "zone of transition" from the "calyptrogen" (or its equivalent) to the outer senescent cells of the root cap. The marked differences in the *rate* and *duration* of

cell division respectively in root cap and the body of the root, present an important but entirely obscure morphogenetic problem.

III. The Vascular Cambium.—The term "vascular cambium" is applied to vertical strips or narrow cylinders of enlarging and dividing cells which are *lateral* in position and which give rise to the secondary phloem and secondary xylem tissue-systems. The vascular cambium is properly regarded as a "secondary meristem" since its activity is responsible for the *addition*, at some distance from the apex of root or shoot, of new or secondary vascular tissues to the original or "primary" conducting system which in turn had its origin in the *provascular meristem* or "procambium." In many herbaceous angiosperms, especially many of the monocotyledons, and in most of the lower vascular plants, cambial activity is reduced or absent and the vascular system is therefore largely "primary" in character. But in woody angiosperms and in the gymnosperms, the primary tissues of stem and root are short-lived and become destroyed or buried by the more massive secondary vascular system formed by the cambium.

The most significant of modern studies on the structure and growth of the cambium have been made by Bailey (1920, 1923, 1930), who has studied both fixed as well as living cells in a wide range of gymnosperms and angiosperms. From a morphological standpoint, the cambium may be regarded as a *single layer* of cells in which *tangential* (i.e., periclinal) *divisions* predominate during the propagation of phloem or xylem. Two principal types of *initials* occur in the cambium, viz.: (1) the *fusiform initial*, which as seen in *tangential* longi-sectional view is prosenchymatous in form and in certain plants, according to Bailey, may attain the enormous length of 5,000μ, and (2) the *vascular-ray initial*, which is a much smaller cell and is more or less isodiametric in form. The fusiform initials form such elements as tracheids, vessels, fibers, wood-parenchyma, and sieve-tubes, while the ray initials are points of origin and propagation of the radially-disposed phloem and xylem rays (cf. Barghoorn, 1940). One of the many interesting features of cambial cells is their highly vacuolate character, which is only evident when liv-

ing tissue is critically studied with the aid of such vital stains as "Neutral Red." Bailey (1930, p. 677) states: "Normal cambial initials are conspicuously vacuolated. Indeed certain of them are as highly vacuolated as plant hairs, which are commonly cited as illustrations of extreme specialization of the protoplast in fully differentiated cells. The classical conception of non-vacuolated meristems, and the various physiological generalizations that have been deduced therefrom should be abandoned." Just how the form, wall structure, vacuome, and peculiar methods of cytokinesis in cambial initials are related to the derivation *in opposite directions* of such *heterogeneous tissue systems* as phloem and xylem is not yet clear. It would seem evident, however, that here, as with comparable problems at the root and shoot apex, experimental studies (e.g., tissue cultures and transplantation) may ultimately illuminate much of the obscurity of this important problem.

The most instructive and realistic views of the vascular cambium are secured from a study of living material which may be stained with neutral red (cf. Appendix, p. 142). With the aid of a sharp, heavy knife and a sliding microtome, it is possible to obtain useful tangential, radial, and transverse sections of the cambium and its recent phloem and xylem derivatives. The cambium of *Pinus* is a good gymnospermous type with greatly elongated *non-stratified* fusiform initials while *Robinia* illustrates a dicotyledonous type with shorter, rather evidently *stratified* fusiform initials.

IV. Suggested Drawings and Notes.—

1. Prepare *outline drawings* of various available types of angiospermous and gymnospermous shoot apices. In each outline, indicate diagrammatically by means of legends, the position and extent of each of the following *when* it occurs: tunica, corpus, provascular strand, rib meristem, pith, cortex, leaf primordium, axillary bud primordium, peripheral tissue zone (in apex of gymnosperms), central tissue zone (in apex of gymnosperms), initial zone.

2. Prepare an outline drawing of an angiospermous shoot apex, filling in all the cells of the tunica and corpus zones and

of the youngest leaf primordia. Also show the form and arrangement of the cells (1) in a small portion of a provascular strand, and (2) at several successively older regions of the rib meristem. Label carefully all important structures.

3. Prepare an outline drawing of *one* of the available types of angiospermous root apices, showing diagrammatically and labelling the following: root cap, subterminal meristem, region of elongation, region of maturation, epidermis, cortex, stele.

4. Select *one* of the types of root apices and fill in the cellular details of the root cap, the calyptrogen (if present) and the so-called "histogens" of the root proper, i.e.,: "dermatogen," "periblem," "plerome." Label all parts of the drawing.

5. Prepare drawings of the cambium of *Pinus* and *Robinia* showing its appearance as seen in the transverse, radial and tangential planes of section. In the drawings of transverse and radial sections, a small portion (i.e., several cell layers) of the adjacent phloem and xylem tissue should be shown.

REFERENCES

1. Bailey, I. W., The Cambium and Its Derivative Tissues, III. A reconnaissance of cytological phenomena in the cambium. Amer. Jour. Bot. 7:417-434. 1920.
2. ————, ——, The Cambium and Its Derivative Tissues, IV. The increase in girth of the cambium. Amer. Jour. Bot. 10:499-509. 1923.
3. ————, ——, The Cambium and Its Derivative Tissues, V. A reconnaissance of the vacuome in living cells. Zeitsch. für Zellforch. u. mik. Anatomie. 10:651-682. 1930.
4. Ball, E., Microtechnique for the Shoot Apex. Amer. Jour. Bot. 28:233-243. 1941.
5. Barghoorn, E. J., Jr., Origin and Development of the Uniseriate Ray in the Coniferae. Bull. Torr. Bot. Club 67:303-328. 1940.
6. Bloch, R., Wound Healing in Higher Plants. Bot. Rev. 7:110-146. 1941.
7. Boke, N. H., Histogenesis and Morphology of the Phyllode in Certain Species of *Acacia*. Amer. Jour. Bot. 27:73-90. 1940.
8. ————, ——, Zonation in the shoot apices of *Trichocereus spachianus and Opuntia cylindrica*. Amer. Jour. Bot. 28: 656-664. 1941.
9. Bower, F. O., Size and Form in Plants. London, 1930.

10. Cross, G. L., The Morphology of the Bud and the Development of the Leaves of *Viburnum rufidulum*. Amer. Jour. Bot. 24:266-276. 1937.
11. ———, ——, The Structure of the Growing Point and the Development of the Bud Scales of *Morus alba*. Bull. Torr. Bot. Club 63:451-465. 1936.
12. ———, ——, The Structure and development of the Apical Meristem in the Shoots of *Taxodium distichum*. Bull. Torr Bot. Club 66:431-452. 1939.
13. ———, ——, Some Histogenetic Features of the Shoot of *Cryptomeria japonica*. Amer. Jour. Bot. 28:573-582. 1941.
14. Eames and MacDaniels, Ch. III.
15. Foster, A. S., A Histogenetic Study of Foliar Determination in *Carya Buckleyi* var. *arkansana*. Amer. Jour. Bot. 22:88-147. 1935.
16. ———, ——, Leaf Differentiation in Angiosperms. Bot. Rev. 2:349-372. 1936.
17. ———, ——, Structure and Growth of the Shoot Apex in *Ginkgo biloba*. Bull. Torr. Bot. Club 65:531-556. 1938.
18. ———, ——, Structure and Growth of the Shoot Apex of *Cycas revoluta*. Amer. Jour. Bot. 26:372-385. 1939a.
19. ———, ——, Problems of Structure, Growth and Evolution in the Shoot Apex of Seed Plants. Bot. Rev. 5:454-470. 1939b.
20. ———, ——, Further Studies on Zonal Structure and Growth of the Shoot Apex of *Cycas revoluta*. Amer. Jour. Bot. 27:487-501. 1940.
21. ———, ——, Comparative Studies on the Structure of the Shoot Apex in Seed Plants. Bull. Torr. Bot. Club. 68: 339-350. 1941a.
22. ———, ——, Zonal Structure of the Shoot Apex of *Dioon edule*. Amer. Jour. Bot. 28:557-564. 1941b.
23. Haberlandt, Ch. II.
24. Hanstein, J., Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen. Festschr. Niederrhein. Ges. Natur. Heilkunde: 109-143. 1868.
25. Hayward, pp. 12-15; 44-47; 63-67; 77-85.
26. Johnson, M. A., Structure of the Shoot Apex of *Zamia*. Bot. Gaz. 101:189-203. 1939.
27. Louis, J., L'ontogénèse du système conducteur dans la pousse feuillée des Dictoylées et des Gymnospermes. La Cellule 44: 87-172. 1935.
28. Priestley, J. H., and Scott, L. I., An Introduction to Botany. London, 1938.

29. Rüdiger, W., Die Sprossvegetationspunkte einiger Monokotyledonen. Beitr. Biol. Pfl. 26:401-443. 1939.
30. Schmidt, A., Histologische Studien am phanerogamen Vegetationspunkten. Bot. Archiv. 8:345-404. 1924.
31. Schüepp, O., Meristeme Handbuch d. Pflanzenanatomie. IV. 1926.
32. Sinnott, E. W., and Bloch, R., Division in Vacuolate Plant Cells. Amer. Jour. Bot. 28:225-232. 1941a.
33. ————, ————, ——, The Relative Position of Cell Walls in developing Plant Tissues. Amer. Jour. Bot. 28:607-617. 1941b.
34. Von Guttenberg, H., Der primäre Bau der Angiospermenwurzel. Handb. d. Pflanzenanatomie. VIII. Berlin. 1940.
35. ————, ——, Der primäre Bau der Gymnospermenwurzel. *Ibid.*, Berlin. 1941.
36. Wolff, C. F. Theoria generationis. Halae, 1759.
37. Zirkle, C. Vacuoles in primary meristems. Zeitsch. für Zellforsch. u. mik. Anatomie. 16:26-47. 1932.

Exercise IV

PROBLEMS IN THE CLASSIFICATION OF CELL TYPES, TISSUES, AND TISSUE SYSTEMS IN VASCULAR PLANTS

I. Introduction.—Despite the vast amount of information which has accumulated during the present century regarding the cellular structure of higher plants, the nomenclature and classification of cell types and "tissues" is still in a confused and uncertain state. Doubtless much of the difficulty arises from the absence in plants of the high degree of structural and physiological individuality which characterizes the tissues of higher animals. For example, certain so-called "permanent" or "adult" tissues, such as parenchyma, collenchyma, and epidermis, may "secondarily" revert to a meristematic state and produce tissues or structures quite different from themselves. Furthermore, it is difficult or even impossible to draw a clear morphological demarcation between adjacent "permanent" tissues in many instances. A good example is furnished by the gradual intergradation between collenchyma and parenchyma tissue in the cortex of many stems. As a consequence of these and other difficulties, the term "tissue" in Plant Histology has been used, sometimes in a broad sense, sometimes in a restricted sense, depending upon the relative importance attributed to position, origin, structure, or function. Sachs (1875, p. 68) adopted in the first place a broad concept by stating that "in the widest sense every aggregate of cells which obeys a common law of growth (usually, however, not uniform in its action) may be termed a tissue." A similar idea is found in Strasburger's Textbook (1921, p. 41), where a tissue is defined as a "continuous aggregation of cells in intimate union." Other definitions of "tissue" are less general in character and introduce, in various ways, the ideas of origin, specific cell structure, and function. For example, Eames and MacDaniels (1925, p. 50) define a tissue

as "a continuous organized mass of cells, usually similar in origin, and essentially alike in form and general function." Hayward (1938, p. 11) adopts a similar attitude by contending that "strictly defined, a *tissue* is a group of cells of common origin having essentially the same structure and performing the same functions." In these definitions, *community of origin*, *continuity* and *similarity in structure* and *function* are essential attributes of the cells of any "tissue." Haberlandt (1914, pp. 56-72), in contrast, has approached the problem from quite a different point of view. He assumes that the differentiation of specific cell-aggregates or "tissues" in plants is "mainly the outcome of division of labor, and that consequently the most characteristic features of each tissue are those which are most intimately connected with its physiological activity." Haberlandt's concept is thus essentially physiological or functional, and the ontogenetic and phylogenetic aspects of tissues are more or less completely disregarded.

It is necessary to emphasize that the wide differences in the concepts of "tissues" outlined above have much more than an historical significance. On the contrary, these differences underlie the varied schemes of tissue classification found in modern botanical texts and continue to influence the study of comparative histology and anatomy. For these reasons, the following critical resumé of the most important early as well as recent schemes of tissue classification is offered as a guide for the student in the interpretation and evaluation of modern histological literature. In addition, it is hoped that this critique may serve to emphasize the need for a complete re-examination of the fundamental assumptions upon which these attempted classifications inevitably rest.

II. Systems of Tissue Classification.—

1. *Sach's classification.* Sachs assumed that, in the phylogenetic development of the higher plants from simple multicellular forms, a distinction gradually arose between the outer layers of cells or "tissue" and the internal mass. The latter finally differentiated strands of cells surrounded by "fundamental" tissue. The final result is seen in the "primary" structure of the leaf, stem, and root, which consist, according to Sachs

(1875, pp. 77-78), of *three* principal *Systems of Tissues*, viz.: (1) the *Epidermal System*, under which are grouped the epidermal and cork layers; (2) the *Fascicular System*, which consists of the variously-arranged xylem and phloem; and (3) the *Fundamental Tissue System*, which Sachs defined as "those masses of tissue of a plant or of an organ which still remain after the formation and development of the epidermal tissue and the fibro-vascular bundles." Sachs (*op. cit.*, p. 103) emphasized that his classification was not concerned basically with the *forms* of cells "but with the contrast of different systems of tissue, each of which may contain the most various cell-forms." It is lastly of interest to note that Sachs did not attempt to demarcate rigidly "permanent" and "meristematic" tissue but on the contrary emphasized the ability, especially of the epidermal and fundamental systems, to regress to the state of a "formative" or dividing tissue.

Sachs' classification of the adult vegetative tissues of higher plants into three main groups is appealing in its apparent simplicity and practicability. That it does indeed possess considerable pedagogical value is shown by its adoption (with or without minor changes) in such modern texts as Sinnott (1935) and Molisch (1936). From a more technical standpoint, Sachs' classification is also used by Jeffrey (1917, pp. 8-13), who emphasizes, however, that the structural boundaries between the three tissue systems are more evident in lower than in higher vascular plants. Furthermore, the limits of the systems appear less sharply defined in the stem than in the "more conservative" leaf and root. In organs where secondary growth is prominent, the boundaries between the vascular and fundamental systems may disappear and "in such cases the limits of the tissues can only be inferred from comparative and developmental anatomy." The chief objection which has been repeatedly raised against Sachs' classification is concerned with the indefinite physiological as well as structural characterstics of the "Fundamental Tissue System." In some organs, this system may consist largely of parenchyma, but many other cell types or tissues may be present. The fundamental tissue system, as Haberlandt (1914, p. 712) states, includes "green photosynthetic parenchyma, colorless water-tissue,

storage-parenchyma, mechanical strands and cell-masses, endodermal layers, and the multifarious tissues which make up pericarps and seed coats. No one, therefore, will venture to maintain that "ground-tissue" constitutes a "whole of definite physiological character."

2. *Haberlandt's classification.* Probably no scheme for classifying plant tissues has been carried out so consistently from a single point of view or in such detail as Haberlandt's "Anatomico-Physiological Classification." According to Haberlandt's viewpoint, the "principal function" should be the *sole guide* in the designation of any specific tissue "system." The "principal function" of a tissue is defined as "that form of physiological activity with which its most obvious and important anatomical features are correlated." The application of this idea resulted in the distinction by Haberlandt (1914, pp. 71-72) of twelve "anatomico-physiological tissue systems," each of which is typified by one major or "principal" function: e.g., absorption, conduction, protection, support, etc. With reference to the merits of his scheme of classification, he contends that "the anatomico-physiological definition and arrangement of tissues provides the broadest and most natural of all systems of tissue classification, since from this point of view the plant-body is regarded not merely as a more or less complex aggregate of formal elements, but also as a living organism, composed of a number of functional units and engaged in a corresponding number of physiological activities, which all contribute to the safety and welfare of the whole."

Haberlandt's high estimate of the value of his method for classifying tissues has been amply justified by its wide adoption in elementary as well as more advanced treatises on plant histology. Tschirch (1889) and Palladin (1914), for example, follow Haberlandt's system with little modification and Molisch (1936) champions its merits for the advanced student. In this country, the anatomico-physiological classification has likewise proved popular and is utilized, in a somewhat simplified form, in such a recent compendium as Hayward (1938). But one of the most significant illustrations of the prestige and influence of Haberlandt's ideas and classification is furnished by the ambi-

tious "Handbuch der Pflanzenanatomie" which treats of the varied phases of anatomy in monographic form. Linsbauer, as the original editor, states in the first volume of this encyclopaedic work, that, aside from certain disagreement in details, the principles of Haberlandt's physiological anatomy will be adopted. In this same volume, an able and penetrating discussion of the various concepts and classifications of tissues is given by Lundegårdh (1922). This author, while agreeing in principle with the anatomico-physiological method of classification, emphasizes the need for a cautious and critical approach to the problem, since "the physiological-anatomical systems only indicate the normal combination of structure and function and obviously do not permit of any teleological conclusions as to the method of their origin." Lundegårdh (*op. cit.*, p. 175) proposes the following anatomico-physiological conspectus of tissue systems, viz.:

I. The Coherent Tissue Systems (composed of continuous cell aggregates).
   A. The Formative or Meristematic Tissues.
   B. The Mature Tissues.
      1. Systems with *dynamic functions*, e.g., assimilation, respiration, storage, absorption, etc.
      2. Systems with *static functions*, e.g., protection, mechanical support, etc.

II. The Disperse Systems (composed of *isolated* cells or cell-groups distributed as "islands" in the midst of various "coherent systems").
   A. Stomata (i.e., guard and accessory cells).
   B. Organs of Perception.
   C. Reproductive Apparati.
   D. Idioblasts (e.g., isolated stone cells found in the mesophyll of certain foliage leaves such as *Camellia*).

Two principal objections have been advanced against Haberlandt's scheme of classification and the fundamental assumptions upon which any anatomico-physiological system is based. First of all, Haberlandt's system is constructed with respect to the nature of the "principal function" of each tissue system. How-

ever, many tissues or cell types carry on more than a single function. In such instances, a distinction between "principal" and "subsidiary" function appears somewhat arbitrary. In other words, certain types of cells might with equal justification be classed in more than one of the anatomico-physiological "systems." Furthermore, as Lundegårdh admits, the principal function of a tissue (e.g., storage of reserve starch) can only rarely be *deduced* from observation only. Hence, any anatomico-physiological classification has a provisional character and is destined to be changed or modified in the light of new experimental data.

In the second place, the objection is raised that in such a scheme as Haberlandt proposes, confusion results because of the disregard of the origin of cells and tissues. For example, in Haberlandt's classification, epidermal and cork cells, although differing fundamentally in origin, are grouped for physio-topographical reasons under the "Dermal" or protective system. Conversely, guard cells and root hairs, while having a common origin from the embryonic surface cell-layer or "protoderm," are classified because of functional differences in the "Ventilating" and "Absorbing" systems respectively. In short, as Haberlandt (*op. cit.*, p. 70) emphasizes, to the physiological anatomist "the homologies of tissues are of no interest . . ." in defining and classifying the various tissues of the plant body; ". . . his concern is solely with analogy." Whether such a viewpoint leads to a "natural" insight into the evolutionary development of plant tissues is open to serious question. Jeffrey (1917, p. 8) says in this regard that "from the point of view of the doctrine of descent, functional features are of less significance, since it is precisely these which are the most readily modified and as a consequence furnish the least valuable indications of the course of evolutionary development in any given large group."

3. *Eames' and MacDaniels' classification.* In contrast to the schemes of Sachs and Haberlandt, Eames and MacDaniels base their classification of tissues on *method of development.* From this standpoint, tissues "which are developed directly or indirectly at the growing points by cell division in several or many planes" are termed *primary tissues.* On the other hand, tissues which "are formed largely by cell division in a single plane,

individual cells consecutively forming many new cells, which because of this method of formation lie in definite rows," are designated as *secondary tissues.* They originate from *cambia* of various types (e.g., the vascular cambium and the cork cambium). This ontogenetic viewpoint is based on the idea that since "parenchyma" is phylogenetically the primitive tissue, *meristem,* which is likewise "unspecialized" and "parenchyma-like," constitutes the natural foundation upon which to base a classification of adult specialized tissues. This ontogenetic scheme of classification is very useful in emphasizing the difference between the "primary" and "secondary" growth and structure of the stem and root in gymnosperms and many dicotyledons. But, from the point of view of cell structure, there is often little or no morphological difference between certain cell types common to both primary as well as secondary tissues. Thus, for example, fibers which differ little in form or structure occur in the cortex and pericycle ("primary tissue" regions) and in the secondary phloem.

Eames and MacDaniels further attempt to subdivide "permanent" tissues into two main groups, viz.: (1) *simple tissues,* such as parenchyma and collenchyma, which consist of a single cell type and are thus *structurally homogeneous:* and (2) *complex tissues,* such as xylem and phloem, which consist of several distinct types of cells and hence are *structurally heterogeneous.* Such a distinction appears to have a very restricted *practical* value, although it may be theoretically justifiable on *phylogenetic grounds.* First of all, very few of the cell types present in higher vascular plants occur as "simple tissues." Parenchyma, it is true, is often "homogeneous," but not infrequently *idioblasts,* in the form of branched sclereides, are scattered among this "tissue." From Lundegårdh's standpoint, these idioblasts would collectively compose a separate "diffuse tissue system." Furthermore, the elements of a "simple tissue" (e.g., fibers or parenchyma cells) may likewise be present as components of a "complex tissue" (e.g., "phloem parenchyma," "phloem fibers," "xylem parenchyma," "xylem fibers").

The preceding critical resumé has attempted to point out briefly the advantages as well as the apparent defects of certain

outstanding schemes of tissue classification. Future progress may be expected when our insight into the *developmental* and *functional potentialities* of the various types of cells has been increased. It seems clear that the fields of *pathological* and *experimental plant anatomy* are destined to contribute largely to a more natural grouping of cells and cell aggregates. Weber (1929), who has characterized all previous anatomy as "cell wall anatomy," contends that a firm basis for the distinction of cell types and tissues must depend upon a better knowledge of protoplasm, with less attention to the morphology of dead and fixed cell walls. In his view, this requires the careful observational and experimental investigation of living protoplasts, which may be "physiologically" distinct although "morphologically" identical. This new approach, which Weber terms "Protoplasmic Plant Anatomy," is still in its infancy, but undoubtedly a better knowledge of structure will appear as our knowledge of the behavior and potentialities of living cells and cell groups increases [cf. the reviews by Bloch (1941) and White (1941)].

Since all methods for classifying plant tissues are open to objection, the writer has adopted a non-committal and "practical" attitude in this book. Instead of following any one scheme of classification, the emphasis is placed first of all upon the salient morphological features of the *principal types of plant cells*. These cell types recur in various regions, "tissues" and organs of the higher plants, and a thorough knowledge of their form, structure, development, and presumable function(s) must constitute the necessary analytical approach to anatomy. If such knowledge is gained through practical laboratory studies, the student should be in a position to study with some degree of independence the comparative anatomy of such organs as the stem, root, and leaf.

In the appended table an effort is made to summarize the important features of the main types of plant cells. Reproductive cells (spores and gametes) as well as specialized secretory or sensitory cells have been deliberately omitted. No pretense of "completeness" is therefore made, but it is hoped that the table may serve its purpose as a basis for the analytical study of plant structure.

SUMMARY OF MAIN CELL TYPES IN SEED PLANTS

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Primordial Meristem	Lineal descendents of cells of embryo.	Apex of shoot and beneath inner edge of root-cap; apex and margins of young foliar organs.	± isodiametric; prominent nucleus; wall thin or irregularly thickened; cytoplasm vacuolated to varying degrees; mitochondria, plastids and storage products may be present.	The point of origin of all primary tissue; in shoot, tissue from which foliar organs develop.
Vascular Cambium	From the procambium or from regressive differentiation of parenchyma.	*Lateral* in stem and root, between phloem and xylem (fascicular) or between vascular bundles (interfascicular).	Two types of initials, viz.: *fusiform*, elongate in form and *ray*, ± isodiametric. In both, cytoplasm highly vacuolated; storage products may be present.	Produces the secondary phloem, secondary xylem and the interfascicular parenchyma.
Cork Cambium (phellogen)	± obvious regressive differentiation of epidermal, collenchyma or parenchyma cells.	Near surface of plant organs.	± short, prismatic initials; apparently highly vacuolated.	Produces cork (phellem) centrifugally and smaller amount of phelloderm centripetally.

SUMMARY OF MAIN CELL TYPES IN SEED PLANTS (*Continued*)

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Epidermal	Protoderm	Surface cells of foliar organs and of young stems and roots.	Variable in shape; guard cells often reniform; outer walls usually cutinized and overlaid by cuticle; protoplast active, vacuolated and often with pigments in cell sap.	Mechanical protection; restriction of transpiration; aeration by means of stomata; storage of water and products of metabolism; concerned in regeneration.
Parenchyma	Ground meristem and vascular cambium.	Widely distributed throughout plant body; may constitute bulk of cortex, pith and mesophyll; present in phloem and xylem.	Vary from approximately isodiametric to cylindrical; primary wall relatively thin, simply pitted and of cellulose; protoplast active and with marked ability for regressive differentiation.	Photosynthesis; food and water storage; conduction; prominently concerned in wound healing and regeneration.
Collenchyma	Ground meristem of leaf and stem.	Hypodermal cylinder or separate strands in cortex of stem and in petiole and ribs of leaves.	± elongate, prismatic, with irregularly thickened primary walls; walls are simply pitted, of cellulose or with alternating lamellae of pectin and cellulose; and contain high % of H_2O; protoplast active and capable of regressive differentiation. Chloroplasts may be present.	Support and elasticity of young stems and of leaves; regeneration.

SUMMARY OF MAIN CELL TYPES IN SEED PLANTS (*Continued*)

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Sclereide	In surface layer of seed coat from the protoderm; elsewhere from secondary sclerosis of parenchyma cells.	Diffused in cortex, pericycle, phloem, pith and mesophyll in groups or as solitary cells; constitute principle cell type in shells of nuts, seed coats, and hard endocarps; prominent in bark.	± isodiametric, columnar or elaborately branched; secondary wall massive, lignified and usually with ramiform pits; protoplast usually absent at maturity.	Produces hard "incompressible" texture and thus furnishes mechanical protection.
Fiber	Protoderm, ground meristem and vascular cambium.	Cortex, pericycle, phloem and xylem; may occur as massive hypodermal strands in foliar organs.	Typical example of prosenchymatous cell; often attaining considerable length; secondary wall usually thick, of pure cellulose or lignified to variable degree; pits vestigial; lumen continuous, ± occluded or may be divided by transverse septa; protoplast usually absent at maturity.	Support and flexibility.
Tracheid	Procambium and vascular cambium.	Xylem	Prosenchymatous; secondary wall lignified and deposited as rings, spiral bands, bars, or a reticulum; or else continuous except for pits; protoplast absent at maturity.	Conduction of water and certain solutes; support.

SUMMARY OF MAIN CELL TYPES IN SEED PLANTS (*Continued*)

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Vessel Element	Procambium and vascular cambium.	Xylem, occurring in form of vertical cell series each of which is termed a vessel.	Form ranges from prosenchymatous to cylindrical; ends of element with simple or scalariform perforations; lateral secondary walls lignified, with same types of patterns as tracheid; protoplast absent at maturity.	Conduction of water and certain solutes; support.
Sieve-tube Element	Procambium and vascular cambium.	Phloem; in angiosperms usually in lateral connection with companion cells and occurring in vertical cell series, each of which is termed a sieve tube.	Form ranges from slender prosenchymatous elements to cylindrical cells; end and often lateral walls provided with sieve plates through which extend cytoplasmic strands; nucleus disappears as cell differentiates.	Conduction of organic solutes.
Cork	Cork cambium (phellogen)	Peripheral regions of stem and root; occasionally developed in foliar organs. Cork tissue impervious to diffusion of gases except through lenticels.	Tabular, compactly arranged with suberized and usually unpitted walls; tannins and crystals may occur; protoplast absent at maturity.	Mechanical protection and restriction of transpiration.

REFERENCES

1. Bloch, R., Wound Healing in Higher Plants. Bot. Rev. 7:110-146. 1941.
2. Eames, A. J., and MacDaniels, L. H., An Introduction to Plant Anatomy. New York, 1925.
3. Haberlandt, G., Physiological Plant Anatomy. Trans. of 4th German Ed. London, 1914.
4. Hayward, H. E., The Structure of Economic Plants. New York, 1938.
5. Jeffrey, E. C., The Anatomy of Woody Plants. Chicago, 1917.
6. Lundegârdh, H. Zelle und Cytoplasma. Handb. d. Pflanzenanatomie. I. Berlin, 1922.
7. Molisch, H., Anatomie der Pflanze. Jena, 1936.
8. Palladin, W. I., Pflanzenanatomie. Trans. of 5th Russian Edition. Leipzig u. Berlin, 1914.
9. Sachs, J., Text-Book of Botany. Oxford, Clarendon Press, 1875.
10. Sinnott, E. W., Botany, Principles and Problems. New York, 1935.
11. Strasburger's Textbook of Botany. 5th English Ed. London, MacMillan and Co., Ltd., 1921.
12. Tschirch, A., Angewandte Pflanzenanatomie. Wien u. Leipzig, 1889.
13. Weber, F., Protoplasmatische Pflanzenanatomie. Protoplasma 8:291-306. 1929.
14. White, P. R., Plant Tissue Cultures. Biol. Rev. 16:34-48. 1941.

Exercise V

THE EPIDERMIS

I. Introduction.—From a purely topographical viewpoint, the term *epidermis* may be applied to the superficial layer of cells in young stems and roots, and in foliar structures. Since the epidermis represents, in this sense, the point of *direct contact* between the plant and its external environment, it is not surprising that this "tissue" exhibits considerable diversity in its structure and functions. Haberlandt (p. 102) has proposed a restricted physiological definition of the epidermis which would include only "those superficial cells or cell-layers, the histological features of which clearly indicate that their principal function is that of a primary tegumentary or dermal system." According to this viewpoint, absorbing hairs and stomata would be excluded on physiological grounds from the epidermis. But as Linsbauer (1930, pp. 4-5) has clearly pointed out, it seems hardly justifiable to place the chief emphasis on the function of "protection" in the definition of the term epidermis. On the contrary, the cells which are morphologically a part of the epidermis may perform varied functions, important among which are mechanical protection, restriction of transpiration, water storage, aeration, storage of various metabolic products, absorption and photosynthesis. To subdivide such a "continuous" layer as the epidermis into various "anatomico-physiological systems" is more likely to result in confusion than is the retention of the broader topographical-morphological concept expressed above. The ontogenetic development of the epidermis likewise justifies its interpretation as a "morphological unit," since its origin is traceable to an external embryonic layer or "protoderm." In lower vascular plants and in many gymnosperms, the protoderm of the shoot appears some distance from the summit of the apex as a superficial layer derived from the periclinal division of the segments of the apical initial or initials. But in many angio-

sperms the protoderm is *directly continuous* with the outermost tunica layer or "dermatogen" of the shoot apex (for further details, cf. Exercise III). In roots, the protoderm likewise is demarcated from the internal meristems in the vicinity of the apex. Hayward (pp. 45-47) has recently summarized the more important "types" of root apices from the standpoint of the way in which the protoderm ("dermatogen") is related to the development of root cap, cortex and stele.

In the majority of seed plants, the epidermis is a uniseriate layer of cells which clothes the "primary body." Aside from the epidermal appendages or *trichomes*, which will be studied later in the exercise, the common cell types composing this layer are *epidermal cells* and the *guard cells*. The epidermal cells, although exhibiting considerable variation in size, shape and arrangement, are usually closely joined with one another, thus forming a sheet of cells which is pierced only by the *intercellular spaces* or pores found between the guard cells. An exceptional type of epidermis occurs in petals, however, since here intercellular spaces are found between ordinary epidermal cells. According to Eames and MacDaniels (pp. 284-285) these "spaces do not open to the outer air, however, since they are covered in all cases by the cuticle." Epidermal cells are roughly "tabular" in form and especially in the laminae of dicotyledonous foliage leaves, have a characteristic *undulate contour* when seen in surface view. A protoplast is normally retained and a great variety of ergastic substances such as anthocyanin pigments, tannins and oils occur in the cells. Epidermal cells exhibit to a notable extent an ability for *regressive differentiation*. This is shown not merely by the origin in certain plants of the phellogen in this layer (Eames and MacDaniels, p. 210) but especially by the important role of epidermal cells in the production of adventitious bud-primordia (cf. Crooks, 1933, Naylor and Johnson, 1937, McVeigh, 1938, and Naylor, 1940).

With the exception of roots and the submersed portions of aquatic plants, the outer walls of the epidermis are covered by a sheet of waxy material which is termed the *cuticle*. This waxy layer is continuous, except for the stomatal openings, and serves to restrict the loss of water from plant organs. The thickness of

the cuticle is highly variable, in some organs being a hardly perceptible "film" while in other instances (e.g., fruits and certain types of leaves) it is extremely prominent (Eames and MacDaniels p. 37, Fig. 24). The *walls* of epidermal cells vary in their structure and chemical composition. Typically, the *outer tangential wall* directly beneath the cuticle, is the most heavily thickened of all the walls and the *inner-tangential wall* the thinnest. Often the *radial wall* tapers in thickness towards the inner tangential wall. *Simple pits* are common in the radial and inner walls of epidermal cells. According to Haberlandt (p. 102) the innermost zone of the outer wall usually consists of "unaltered cellulose" and is followed externally by layers of wall substances which contain varying amounts of *cutin*. The recent work of Anderson (1934), however, has shown that in the leaf of *Clivia nobilis*, the thick *outer wall* of the epidermal cells shows "two distinct zones of cutinization." The outermost zone is devoid of cellulose or pectin while "the inner zone of cutinized wall consists of a series of cellulose lamellae separated by layers of pectic material, both of which are impregnated with cutin. The inner cutinized zone may be in direct contact with the protoplasm of the cell or may be separated from the protoplasm by a second zone of cellulose and pectic materials." It is clear from this work that a thorough study of the process of cutinization in epidermal walls of various plants is urgently needed.

The continuity of the epidermis, especially of foliage leaves and young stems, is interrupted by minute openings or pores which are termed *stomata*. Each stoma represents an intercellular space between a pair of highly specialized epidermal cells known as *guard cells*. As seen in surface view, guard cells are very frequently crescent-shaped with their concave surfaces adjacent to the slit-like pore. In contrast to ordinary epidermal cells, the walls of guard cells are uneven in thickness, often with ridge- or flange-like extensions at the edges of the pore. Furthermore, guard cells usually contain prominent *chloroplasts*. Since stomata play such an important role in the processes of respiration, photosynthesis and transpiration, much attention has been devoted to the "mechanism" by which the stomata are "opened" and "closed." In general, changes in the width of the stoma

are regulated by the relative degree of turgor in the guard cells, which in turn causes slight alterations in their shape. When the guard cells are turgid, the width of the pore is at a maximum while closing of the aperture occurs when the turgor of the cells decreases. A discussion of the variation in the construction of the walls of guard cells and of the physiological factors influencing the turgor movements of these cells, however, is beyond the scope of this book (cf. Haberlandt pp. 445-477). Guard cells originate by the anticlinal division of certain protoderm cells into two dissimilar daughter cells. In the simplest condition, one of these cells functions as an *initial* cell and by an anticlinal division directly produces the two guard cells; the other daughter cell meanwhile differentiates into an ordinary epidermal cell. But many deviations from this simple type of stomatal development occur, especially with reference to the formation of the *subsidiary cells*. The latter differ in form and arrangement from the neighboring epidermal cells and are believed to cooperate physiologically with the guard cells in regulating the width of the stoma (for information regarding the various modes of stomatal development cf. De Bary, pp. 39-45, Porterfield, 1937, and Yarbrough, 1934).

A study will be made first of the structure of the uniseriate epidermis. Later in this exercise a brief explanation and directions for study of the *multiple epidermis* and of *trichomes* will be given.

II. Material for the Study of the Uniseriate Epidermis—

1. The bulb-scale of the onion (*Allium Cepa*). Remove, with forceps, a small strip of epidermis from the outer or *abaxial* surface of the bulb-scale and mount it carefully in water. Under low magnification, note the rather orderly arrangement of the "rectangular" or tabular epidermal cells. Stomata, which may be abortive or "abnormal" in appearance, are occasionally present in this material. Under high magnification, it will be seen that the *radial walls* of the epidermal cells are provided with numerous small *simple pits*. Because of the comparatively small radial depth of the epidermal cells, nuclei are readily seen. The cytoplasm is highly vacuolate and often is actively streaming or moving within many of the cells. Small greenish-yellow bodies,

which may represent either *leucoplasts* or ergastic material are also observable. To study the cuticle and the structure of the walls of the epidermal cells, cut thin trans-sections of the bulb-scale, and mount them in a .1% solution of neutral red.[1] If these sections are not overstained, the neutral red will be confined largely to the protoplasts and the walls will be clearly demarcated. Similar trans-sections, when gently heated in a solution of Sudan IV,[2] are particularly suitable for a study of the cuticle which will appear under high magnification as a brilliant red layer continuous over the outer walls of the epidermis.

2. The leaf blade of the geranium (*Pelargonium*) or the "German Ivy" (*Senicio mikanoides*). Remove small strips of the lower (*abaxial*) epidermis and mount some in water, others in a .1% solution of neutral red. Note especially the characteristic undulate contours of the epidermal cells and the numerous stomata. If the geranium epidermis is used, the relationship of the numerous *multicellular hairs* can be readily studied.

3. The leaf of *Iris* sp. Mount strips of the epidermis in water as well as in neutral red and examine carefully under low magnification, noting the regularly-arranged stomata and the elongate form of the epidermal cells. Careful focusing will show that the guard cells with their stomata are slightly sunken beneath the surface and are overarched by the ends of the epidermal cells adjacent to them. Thin trans-sections of the epidermis should also be secured and treated with neutral red and Sudan IV. These sections will reveal the thick cuticle and the prominently thickened outer tangential walls of the epidermal cells.

4. Prepared slides of trans-sections of the leaf-blades of lilac (*Syringa vulgaris*) and corn (*Zea Mays*). Trans-sections cut by hand are often too thick to permit of an accurate examination of the structure of the walls of guard cells. For this reason, a supplementary study should be made of the guard cells as seen in thin stained sections of the forms listed above.

III. The Multiple Epidermis.—In the leaves of certain angiosperms, particularly in members of the families *Piperaceae*, *Begoniaceae* and *Moraceae*, some or all of the cells of the original

[1] Cf. Appendix, p. 142.
[2] Cf. Appendix, p. 142.

uniseriate epidermis may experience tangential divisions which result in the formation of a *multiple* or *multiseriate epidermis*. According to the resumé of the literature given by Linsbauer (1930, pp. 38-43), the first tangential divisions leading to the development of the multiple epidermis occur at a relatively late stage in leaf ontogeny; in *Ficus*, for example, as the leaf is expanding and after its stipules have fallen away. Such divisions apparently occur first near the middle portions of the lamina and progress towards its margins. Often the tangential divisions are so regular as to produce a tissue composed of thin-walled cells arranged in radial rows. But anticlinal divisions may also occur in the cells thus obscuring the point of origin of the tissue. It should be apparent from these facts that the concept of the "multiple epidermis" is based fundamentally upon its direct origin from the surface cell layer and not upon its histology or functions. This is important because much confusion has been produced, especially in the literature of physiological anatomy, by failing to distinguish between a true multiple epidermis and the various types of "hypodermal" tissues which originate and develop independently of the epidermis. The chief *function* of the multiple epidermis is water storage and a brief discussion of its activities in this direction are summarized by Linsbauer (1930, pp. 41-43). According to the earlier work of Pfitzer which is outlined by Linsbauer, water-storing tissue of epidermal origin may in *Peperomia pereskiaefolia* attain a thickness of 14-15 layers and thus represents about 7 times the thickness of the other leaf tissues.

IV. Material for the Study of the Multiple Epidermis.—A typical example of a multiple epidermis is found in the leaf blade of the common "rubber plant" (*Ficus elastica*). As early as 1827, the German botanist Meyen observed that during the formation of the multiple epidermis in this species, certain of the original epidermal cells fail to divide tangentially but instead become enormously distended inwardly and finally protrude into the underlying palisade parenchyma. During this process of distention, a curious peg-like *invagination* develops from the outer tangential wall into the cell cavity and eventually becomes

coated with a crystalline mass of calcium carbonate. This curious ingrowth of the cell wall, together with its covering of calcium carbonate is termed a *cystolith* and the cell in which it occurs is known as a *lithocyst*. (Cf. the recent work of Ajello, 1941.) *Obtain* a trans-section of a portion of a living leaf-blade of *Ficus elastica* and examine it under low and high magnification. The *multiple epidermis* on the *upper* (i.e., *adaxial*) surface consists of several layers of cells the *outermost* of which are small, compact and overlaid by a prominent cutiele. These cells thus exhibit the features of a normal "uniseriate epidermis."

In contrast, the *inner cells* of the multiple epidermis are relatively large and because of their shape, their cellulose walls and the presence of intercellular spaces suggest resemblance to cortical parenchyma tissue. Notice that the cells are not aligned in radial rows because, during their formation, both anticlinal as well as periclinal divisions have occurred. A careful inspection of the walls of these cells under high magnification will reveal numerous *simple pits*. At intervals large sac-like *lithocysts* will be seen, protruding into the adjacent palisade parenchyma. These distended cells have arisen *directly* from the original surface cells of the leaf blade. Each lithocyst, unless injured in sectioning the leaf, contains a *cystolith* with its knob-like end covered by a crystalline mass of calcium carbonate. Introduce a few drops of *hydrochloric acid* under the cover-glass and observe the rapid dissolution of the calcium carbonate. This is accompanied by the evolution of small bubbles of carbon dioxide.

V. Trichomes.—This term may be used in a collective sense to designate the diversified types of epidermal appendages such as *hairs*, *scales*, *colleters* and *water vesicles*. Despite the "endless" variation in the form and structure of trichomes (cf. De Bary, pp. 54-66, and Netolitzky, 1932), these structures *originate* from the extension or subdivision of protoderm cells. Trichomes are therefore, morphologically, a part of the epidermis in contrast to *emergences* (e.g., the prickles on the stem of *Rosa*, *Ribes*, etc.) which consist of cells derived not only from the protoderm but also from deeper hypodermal layers (cf. De Bary, p. 58, and Eames and MacDaniels, p. 1 and p. 2, Fig. 1).

The morphological distinction between trichomes and emergences is of further interest in those cases where hairs or scales are borne upon an emergence (Cooper, 1932).

Trichomes furnish a rich field for morphogenetic investigations because of their great diversity and because their superficial position and relatively simple structure facilitate ontogenetic studies with living material. As an introduction to the problems in this field, a brief characterization of the four commonest "types" of trichomes is now given, viz.:

1. *Hairs.* In form, hairs are thread-like in appearance and are either *unicellular* (e.g., root hairs) or *multicellular.* The latter type of hair may consist of a single series of cells, terminating in an acute terminal cell or a *glandular cell;* or the hair may be branched in various ways. In some plants, the hairs are composed of several layers of cells and are termed *shag-hairs.* Such multiseriate hairs are often borne upon an emergence (De Bary, pp. 64-65, and Fig. 21c). Two general regions may be distinguished in a hair, viz.: (1) the *foot,* which is the portion lying within the epidermal surface and which is often different in form from the adjacent epidermal cells, and (2) the *body* which is the portion extending away from the epidermal surface. Occasionally, a given epidermal surface develops but a single type of hair (e.g., root hairs). More commonly, especially in leaves, several different morphological types of hairs occur side by side on the same epidermal area.

2. *Scales.* These trichomes consist of a plate of cells and are either *peltate* (as in certain angiosperms) or are attached to the epidermis only at one edge (e.g., the chaffy scales or *paleae* so characteristic of many ferns). Scales in the genera *Shepherdia* and *Elaeagnus* are so closely crowded on the surface of the young stem and the leaves that they produce a typical "scurfy" appearance.

3. *Colleters.* On many foliar organs, particularly on bud scales and stipules and on the foliage leaves of certain genera (e.g., *Rhododendron, Carya*) glandular trichomes occur. These structures were originally termed colleters by Hanstein (1868). Colleters consist of a short, often multicellular stalk bearing an expanded disc or knob of *secretory cells.* The characteristic

sticky exudation found on certain foliar structures is secreted from colleters (cf. Foster, 1929, pp. 457-458).

4. *Water vesicles or bladders.* Trichomes of this type consist of greatly distended epidermal cells which presumably are of physiological importance as reservoirs for water (cf. Haberlandt, p. 116). In the so-called "Ice Plant" (*Mesembryanthemum crystallinum*), the water vesicles are so large and so numerous that the leaves and young stems appear to be covered with minute translucent beads of "ice."

VI. Material for the Study of Trichomes.—

1. *Hairs.* Obtain thin trans-sections of the petiole of the geranium (*Pelargonium*) and after mounting them in water,[1] study carefully the structure of the *unicellular* and the *multicellular unbranched* hairs. Note the relatively thick outer walls of the body of these hairs. The foot, especially of the multicellular hair, consists of an enlarged bulbous cell, separated by a transverse wall from the body, and surrounded by a circular group of more or less elevated *subsidiary cells.* The true relationship of the subsidiary cells to the foot of the hair is clearly seen in thick trans-sections of the petiole as well as in strips of epidermis removed from the lower surface of the lamina. Note that certain of the hairs are *glandular*, terminating in a single large secretory cell filled with dense, yellowish-brown ergastic material. Excellent material for a study of *multicellular branched* hairs is afforded by the leaves of various members of the Malvaceae, where typical *stellate hairs* occur. Each hair of this type consists of a number of radiating unicellular "branches" which have arisen from the subdivision of a single epidermal cell. Further illustrations of multicellular branched hairs are provided by the leaves of mullein (*Verbascum Thapsus*). *Scrape* a small amount of hairs from the leaf into water, carefully tease them apart with dissecting needles and examine under low magnification. Note that these complex hairs are "dendroid" in form; i.e., each hair consists of a main "axis" (made of a vertical series of cells) and whorls of radiating unicellular or bicellular "branches." Trans-sections of very young mullein leaves are

[1] Cf. Appendix, pp. 139-140.

very instructive in showing various stages in the ontogeny of these hairs. The leaf-blade of the sycamore or buttonball tree (*Platanus*) likewise will provide examples of dendroid multicellular hairs.

2. *Scales.* Scrape a small quantity of trichomes from the leaf of *Shepherdia* or *Elaeagnus* into a drop of water on a slide and examine under low magnification. In *Shepherdia* two extreme types of peltate trichomes will be seen, viz.: (1) *Stellate hairs*, consisting of a delicate stalk bearing ten or more distinct radiating unicellular "branches," and (2) *Scales* which are gray-yellowish brown in color, lobate and consist of a plate of many cells. Note under high magnification that *not all* of the cells composing the disc-like scale radiate from a common center. According to the recent ontogenetic studies of Cooper (1932) this condition results from *unequal and oblique divisions* which may occur near the outer end of certain of the cells early in the development of the scale.

3. *Colleters.* The bud scales of the horse-chestnut (*Aesculus Hippocastanum*) provide excellent material for a study of typical colleters. Cut thin trans-sections of the inner scales of a winter bud and mount them in water. Under low magnification note that the abaxial surface of the scale in particular is densely covered with colleters. Additional or alternative material for a study of colleters is furnished by the bud scales of various species of *Rhododendron*.

4. *Water vesicles.* Obtain several thin trans-sections of the petiole of the "Ice-Plant" (*Mesembryanthemum crystallinum*) and examine them in water under low magnification. The *adult* water vesicle appears as a large clear hemispherical cell which projects outwardly from the general epidermal surface. Under high magnification, a nucleus, scanty cytoplasm and small plastids may be detectable in the bladders. If the concave adaxial surface of the petiole of immature leaves is examined, various stages in the origin and expansion of the water vesicle may be seen.

VII. Suggested Drawings and Notes.—

1. *The uniseriate epidermis.* Prepare carefully labeled drawings to show the structure, in both surface and trans-sectional

views, of the epidermis of *Allium*, *Pelargonium* (or *Senecio*) and *Iris*. In drawings of the surface view, emphasize the shape and arrangement of the stomata with reference to the epidermal cells. In drawings of trans-sections, indicate clearly the extent and relative thickness of the cuticle, the thickness and pitting of the walls of the epidermal cells and the relation of the epidermal cells to the underlying tissues of the leaf. Also *draw* a single stoma from stained trans-sections of the leaf of *Syringa* or *Zea* to show the structure of the guard cells and the form and extent of the substomatal cavity.

2. *Multiple epidermis.* Prepare a large drawing to show in detail the structure of the multiple epidermis of the leaf of *Ficus elastica*. Include in this drawing a lithocyst with its cystolith. Label all essential structures.

3. *Trichomes.* Prepare drawings, to illustrate the structure and relationship to the epidermal layer of various types of *hairs*. If time permits, also prepare drawings of a scale from the leaf of *Shepherdia* or *Elaeagnus*, of colleters as seen in trans-sections of a bud scale, and of the water vesicle of the "Ice-Plant."

4. *Describe*, by means of diagrammatic drawings, the ontogeny of a water vesicle in the petiole of the "Ice-Plant." *Summarize*, from information given in Haberlandt, the most important functions performed by the various types of trichomes.

REFERENCES

1. Ajello, L., Cytology and Cellular Interrelationship of Cystolith Formation in *Ficus elastica*. Amer. Jour. Bot. 28:589-594. 1941.
2. Anderson, D. B., The Distribution of Cutin in the Outer Epidermal Wall of *Clivia nobilis*. Ohio Jour. Science 34:9-18. 1934.
3. Cooper, D. C., The Development of the Peltate Hairs of *Shepherdia canadensis*. Amer. Jour. Bot. 19:423-428. 1932.
4. Crooks, D. M., Histological and Regenerative Studies on the Flax Seedling. Bot. Gaz. 95:209-239. 1933.
5. De Bary, pp. 29-107.
6. Eames and MacDaniels, pp. 107-112.
7. Foster, A. S., Investigations on the Morphology and Comparative History of Development of Foliar Organs, I. The foliage leaves and cataphyllary structures in the horse-chest-

nut (*Aesculus Hippocastanum* L.). Amer. Jour. Bot. 16: 441-501. 1929.
8. Haberlandt, pp. 101-133.
9. Hanstein, J., Über die Organe der Harz-und Schleimabsonderung in den Laubknospen. Bot. Zeit. 26: 697-713 et seq. 1868.
10. Hayward, pp. 15-18.
11. Linsbauer, K., Die Epidermis. Handbuch d. Pflanzenanatomie, IV, 2. 1930.
12. McVeigh, I., Regeneration in *Crassula multicava*. Amer. Jour. Bot. 25:7-11. 1938.
13. Naylor, E. E., and Johnson, B., A Histological Study of Vegetative Reproduction in *Saintpaulia ionantha*. Amer. Jour. Bot. 24:673-678. 1937.
14. Naylor, E., Propagation of *Hyacinthus* by Leaf Cuttings. Bull. Torr. Bot. Club 67:602-606. 1940.
15. Netolitzky, F., Die Pflanzenhaare. Handbuch d. Pflanzenanatomie, IV. 1932.
16. Porterfield, W. M. Jr., Histogenesis in the Bamboo with Special Reference to the Epidermis. Bull. Torr. Bot. Club 64:421-432. 1937.
17. Yarbrough, J. A., History of Leaf Development in *Bryophyllum calycinum*. Amer. Jour. Bot. 21:467-484. 1934.

Exercise VI

PARENCHYMA CELLS

I. Introduction.—The term "parenchyma" is used in a rather abstract or loose sense to designate a wide variety of living cells which occur in many different regions of the plant body. Parenchyma cells may appear in groups scattered among highly specialized conducting elements, as for example the cells of vascular rays and the vertical files of phloem and xylem parenchyma cells. Often, however, parenchyma cells form homogeneous or "simple" tissues which may constitute a large part of the softer regions of leaves, stems, roots and fruits. From these illustrations it should be clearly evident that under the concept of "parenchyma" are included cells which differ markedly in their position, origin and functions. For this reason, "parenchyma" is merely a convenient and long-established anatomical category within which are included cell types which are not necessarily either homologous or analogous.

In an effort to characterize parenchyma tissue more precisely, it is commonly described as being composed of cells essentially isodiametric in form, separated by more or less conspicuous *intercellular air spaces*, and with thin walls and active protoplasts. To appreciate the merits as well as the limitations of this set of characteristics, the following critique will be found useful.

1. *Form of cell.* The recent work of Marvin (1939) and of Marvin and Matzke (1939) has shown that the form of parenchyma cells in some regions (e.g., the pith of *Eupatorium*) is approximately isodiametric and that with respect to the number of faces of contact made with neighboring cells, "the cells show an economy of surface to volume approaching, and in some cases equaling, that of an orthic tetrakaidecahedron or a rhombic dodecahedron of equal volume." This conclusion is particularly interesting in view of Tupper-Carey and Priestley's (1924) statement that the cells of the apical meristem approach the form

of twelve- or fourteen-sided polyhedra. But, not all cells designated as "parenchyma" are isodiametric in shape. As examples, the following may be cited: the narrowly "cylindrical" form of palisade parenchyma cells, the lobed form of "arm-palisade" cells (cf. Meyer, 1923, p. 16) and the elongated shape of the cells in vascular rays.

2. *Structure and chemistry of the wall.* During the differentiation of the parenchyma cells in the cortex and pith of stems and in the mesophyll of leaves, little or no appreciable increase in wall thickness occurs and a true secondary wall, clearly defined from the original primary wall, may be absent. In such cells, the thin primary wall seems to consist largely of cellulose. Simple pits are present but they are often restricted to certain local regions of the wall (cf. De Bary, p. 117, Fig. 46). In contrast, wood-parenchyma cells and the cells of xylem rays are often provided with relatively thick walls, which are abundantly pitted. According to Eames and MacDaniels (p. 68), the parenchyma cells of secondary wood often have "thick, more or less strongly lignified walls." Whether the thick areas of the walls of these cells are "secondary" or "primary" in nature apparently constitutes an open problem at present.

3. *The protoplast.* The retention at maturity of an active protoplast represents one of the most important characteristics of parenchyma cells. Indeed, because of this fact, parenchyma cells perform many of the most fundamental physiological processes, notably photosynthesis, food and water storage and secretion (Meyer, 1923; Netolitzky, 1935; Sperlich, 1939). In addition to their important metabolic activity, however, parenchyma cells possess to an exceptional degree the ability to revert to a meristematic state (Hayward, 1938, p. 14). This is clearly shown by the rapid response of parenchyma tissue to the physical and chemical effects of artificial or natural wounding. The nature of such responses is highly variable and complex, ranging from the production of "callus" or of cork tissue, to the regressive formation of root or bud primordia [cf. Priestley and Swingle (1929) and Bloch (1941)]. It may be argued that the ease with which parenchyma cells can be induced to divide and to produce new tissues and organ primordia is evidence of their "primitive" or

"unspecialized" nature. But it is evident that many factors, hereditary as well as environmental, influence the process of "regressive differentiation" in parenchyma and that our knowledge of the "potentialities" of such tissue is still in an exploratory stage.

II. Material for the Study of Parenchyma.—At this point suggested material for a preliminary study of storage and photosynthetic parenchyma is described. Additional examples of parenchyma are given in several of the later exercises in this book.

1. *Storage parenchyma.* As noted above, parenchyma tissue often serves as a region for storage of many different substances, particularly starch. The cotyledons of bean embryos, during the early stages of seed germination, provide useful material. *Examine* thin sections as well as partially macerated tissue, noting the closely-packed starch grains in the parenchyma cells. Under high magnification, the type and distribution of the *simple pits* on the various faces of the wall may be clearly studied. Additional examples of storage parenchyma are furnished by the cortical parenchyma of the young root (e.g., *Ranunculus*) and the parenchyma tissue of the potato tuber (*Solanum tuberosum*).

2. *Photosynthetic parenchyma.* In the subepidermal region of young stems and in the mesophyll of leaves, the thin-walled parenchyma cells contain chloroplasts and perform the function of photosynthesis. Obtain a thin transverse section of the stem of *Begonia*, and, after mounting it in water, examine the preparation at low magnification. Notice that the cortex (i.e., the region between the epidermis and the cylinder of vascular bundles) and the pith are composed of large, thin-walled "isodiametric" cells. (Note: Several layers of small collenchyma cells are found at the outer edge of the cortex and may be disregarded in this study.) Under low magnification observe that large, solitary *prismatic crystals* as well as druses occur in many of the parenchyma cells. Frequently, the form of the crystals is highly irregular. Under high magnification, the cytoplasm, vacuole and small chloroplasts can be readily studied especially if the sections are mounted in .1% solution of neutral red.[1] Because of the large size of the cells, the nucleus is only seen occasionally in trans-sections of the

[1] Cf. Appendix, p. 142.

parenchyma tissue. Small simple pits may be visible in certain walls of the cells. In order to understand the shape and proportions of the parenchyma cells, as well as the intercommunication between the prominent intercellular spaces, longi-sections of the stem should be studied under low and high magnification. Observe that the intercellular space system in the longi-section appears "black" because of the included air. This optical effect is of great assistance in distinguishing between walls and longitudinally extended air spaces.

III. Suggested Drawings and Notes.—

1. Prepare an enlarged drawing of a single cell from the cotyledon of the bean seedling, showing its shape, wall structure and the included starch grains. For comparison, draw several cells from the cortex of the root of *Ranunculus* and the potato tuber. Prepare notes on the differences in the form and structure of the starch grains in these different examples.

2. Prepare drawings, from both transverse and longi-sections, showing the structure of the photosynthetic parenchyma of the cortex of the *Begonia* stem. Include cells which show crystals, cytoplasm and chloroplasts. What conclusion do you reach as to the shape of the parenchyma cells? Summarize, from the references you have read, the method of origin and functions of intercellular spaces in parenchyma tissue (cf. especially Turrell, 1936).

REFERENCES

1. Bloch, R., Wound Healing in Higher Plants, Bot. Review 7:110-146. 1941.
2. De Bary, pp. 115-119.
3. Eames and MacDaniels, pp. 52-54.
4. Hayward, p. 14.
5. Marvin, J. W., Cell Shape Studies in the Pith of *Eupatorium purpureum*. Amer. Jour. Bot. 26:487-504, 1939.
6. Marvin, J. W., and Matzke, E. B., A New Method for the Construction of Three-dimensional Cell Models. Amer. Jour. Bot. 26:101-103, 1939.
7. Meyer, F. J., Das trophische Parenchym. A. Assimilationsgewebe. Handbuch d. Pflanzenanatomie. IV. 1923.
8. Netolitzky, F., Das trophische Parenchym. C. Speichergewebe. Handbuch d. Pflanzenanatomie. IV. 1935.

9. Priestley, J. H., and Swingle, C. F., Vegetative Propagation from the Standpoint of Plant Anatomy. U. S. D. A. Tech. Bulletin No. 151. 1929.
10. Sperlich, A., Das trophische Parenchym. B. Exkretionsgewebe. Handbuch d. Pflanzenanatomie. IV. 1939.
11. Tupper-Carey, R. M., and Priestley, J. H., The Cell Wall in the Radicle of *Vicia faba* and the Shape of the Meristematic Cells. New Phytol. 23:156-159. 1924.
12. Turrell, F. M., The Area of the Internal Exposed Surface of Dicotyledon Leaves. Amer. Jour. Bot. 23:255-264. 1936.

Exercise VII

COLLENCHYMA CELLS

I. Introduction.—The subepidermal region of many stems, petioles and ribs of leaves is occupied by a more or less "simple" or homogeneous tissue which is termed collenchyma. Frequently this tissue occurs as a continuous hypodermal cylinder but in some stems and in the petioles of leaves, distinct strands of collenchyma may be present. An interesting example of the strand-like arrangement of collenchyma is furnished by the familiar "strings" present in the abaxial ribs of celery petioles (cf. Esau, 1936, pl. 2 and 6).

In certain respects, collenchyma is remarkably similar to cortical parenchyma. Indeed Hayward (p. 22) regards collenchyma as a derived form of parenchyma, a viewpoint also expressed by De Bary (pp. 119-120) who stated that "it is then to a great extent a matter of taste how far one will extend the term Collenchyma." The parenchymatous character of collenchyma is shown by the fact that it is a tissue composed of living cells, the protoplasts of which, like parenchyma, are able to revert to a meristematic state. This feature is illustrated by the origin of the phellogen or cork cambium in the outermost collenchyma cells in many stems. Furthermore, collenchyma, although primarily a "mechanical tissue" in its function, presumably carries on some photosynthesis as evidenced by the frequent occurrence of chloroplasts in its cells. The *shape* of collenchyma cells, especially those adjacent to parenchyma elements in the cortex or petiole, may be more or less "isodiametric." Typically, however, collenchyma cells are elongated, prismatic elements with obtuse, pointed or oblique ends.

The most definitive characteristic of collenchyma cells is found in the *irregular and often massive thickenings* of the cell wall. These wall thickenings, which are deposited by the protoplast in the form of extensive longitudinal strips, vary in their

position according to several definable types of patterns when the cells are examined in trans-section. In perhaps the commonest type, the thickened areas of the wall are largely restricted to the corners where the cells meet. Collenchyma of this type is termed "*angular*." In certain plants, the thickenings are confined to the tangential walls of the cells, resulting in the so-called *lamellar* collenchyma. Ordinarily, intercellular spaces are small or even absent in collenchyma tissue but in certain Compositae the thickened areas of the walls of the collenchyma cells border upon large and prominent air-spaces. The term "*tubular*" has been applied to this type of collenchyma. These "types" of collenchyma, however, are not rigidly distinct, because in some instances transitions from one to the other may occur in successive radial portions of the same zone of collenchyma.

The *structure* and *chemical composition of the wall-thickenings* in collenchyma cells have recently been studied by several investigators. Anderson (1927), in his studies on the angular collenchyma of tomato (*Solanum lycopersicum*), concluded that the thickenings, which have a high content of water, consist of alternating lamellae of cellulose and pectin. When viewed under crossed Nicols, the walls appeared bi-refringent. Esau (1936) found that the walls of collenchyma cells in celery petioles "are chiefly of cellulose and contain a high percentage of water." She interprets the wall thickenings as representing a special development of the primary wall. Simple pits are found in the walls of collenchyma cells but are not necessarily restricted to either the thin or thickened areas.

The thorough study of Esau (1936) has shown that the *ontogeny* of collenchyma tissue in celery petioles exhibits many interesting and important features. In the mature petiole, the collenchyma occurs in the form of distinct strands which correspond in position to the abaxial ribs. The origin of these collenchyma bundles is traceable to the localized periclinal and anticlinal subdivision of ground meristem cells in the young petiole. Procambial-like strands of somewhat elongated, thin walled cells are thereby produced and it is from such cells that the adult collenchyma eventually differentiates. At first the young collen-

chyma cells are relatively small in diameter but as the rate of cell division slows down, the cells expand and gradually acquire the characteristic angular wall thickenings. The relative prominence of intercellular air spaces in the mature collenchyma strand depends in general upon the time of origin of the tissue. Air spaces are only present when the divisions leading to collenchyma development occur in loosely-arranged ground meristem cells.

Functionally, collenchyma tissue provides considerable strength as well as elasticity to young stems and to leaves. According to Esau (1936), the collenchyma of celery petioles mechanically "is much stronger than the vascular tissue. The breaking load of collenchyma may be two to four times that of the entire vascular bundles or the bundle cap."

II. Material for the Study of Collenchyma.—The clearly defined angular collenchyma in the petiole of *Datura stramonium* provides excellent material and its general structure will now be described. (*Note:* If *Datura* is not available, the angular collenchyma in the cortex of the stems of *Solanum lycopersicum*, *Cucurbita*, or *Begonia*, or the collenchyma strands in celery petioles, may be used.)

Obtain a trans-section of the petiole of *Datura* and after mounting it either in water or a .1% solution of neutral red, examine under low magnification. The *epidermis* possesses characteristically thickened outer walls covered by a prominent cuticle; a protoplast and small scattered chloroplasts should be visible in some of the epidermal cells. Note the prominent *multicellular unbranched hairs* and study carefully the structure of their component cells. Beneath the epidermis are found six or more "layers" of typical *angular collenchyma cells*, the irregularly thickened walls of which exhibit, when viewed in water, a characteristic pearly-white lustre. In sections mounted in dilute neutral red solution,[1] the walls are brilliantly stained and their relationships more readily investigated. Select a thin well-cut area in the collenchyma and study the cells under high magnification. The cell cavities have a more or less undulate outline which is the result of the alternation of thin and greatly thickened

[1] Cf. Appendix, p. 142.

areas of the primary wall. These thickened areas will be seen to be restricted largely to the cell-corners, hence the term "angular" collenchyma. Especially in sections stained with neutral red, the wall thickenings exhibit evidence of a lamellated structure. Observe that "spaces," varying greatly in size and distribution, occur throughout the collenchyma tissue. Some of these apparent "spaces" represent the trans-sectional appearance of the narrow tapering ends of collenchyma cells; others are true *intercellular air spaces*. The distinction between these two conditions is best appreciated, however, by a study of longi-sections of the tissue. Note the highly vacuolate cytoplasm and the chloroplasts in the collenchyma cells.

To understand thoroughly the shape of collenchyma cells and the distribution of the thickened areas of their primary walls, examine under low and high magnification longi-sections of the *Datura* petiole. Study carefully the band-like thickenings of the wall and note their relationship in adjacent cells. The intercellular spaces should now be more evident. *Simple pits* may be seen in both face and section views. Observe that individual cells are often subdivided by thin transverse or sloping walls.

III. Suggested Drawings and Notes.—

1. Draw on a large scale a sector of the trans-section of the petiole of *Datura* (or of the substitute material previously listed) about 5 to 6 cells in width, extending from the epidermis to the cortical parenchyma tissue. Label carefully all important structures.

2. Prepare a drawing of a small portion of the collenchyma tissue as seen in longi-sectional view. Show clearly the distribution of the thickened areas of the wall, the pitting and the intercellular spaces. Fill in the contents of a single collenchyma cell.

3. Summarize, in the form of laboratory notes, the evidence discussed by Esau (1936) which shows that the walls of collenchyma cells are rich in water.

REFERENCES

1. Anderson, D., Ueber die Struktur der Kollenchymzellwand auf Grund mikrochemischer Untersuchungen. Sitzber. Akad. Wiss. Wien. Math.—Natur. Kl. 136:429-440. 1927.

2. De Bary, pp. 119-120.
3. Eames and MacDaniels, pp. 54-56.
4. Esau, K., Ontogeny and Structure of Collenchyma and of Vascular Tissues in Celery Petioles. Hilgardia 10:431-467. 1936.
5. Haberlandt, pp. 155-158.
6. Hayward, p. 22.

Exercise VIII

SCLEREIDES

I. Introduction.—From the standpoint of function, two general types of strengthening or "mechanical" tissues are conventionally distinguished, viz.: (1) *collenchyma*, which is composed of living cells and which is the first strengthening tissue to appear in the development of the stem and leaf, and (2) *sclerenchyma* which is made up of thick-walled cells, usually lacking a protoplast at maturity and which represents the "permanent" and more important of the supporting tissues in the older portions of the plant body. From a morphological standpoint, many authors (e.g., Eames and MacDaniels, Hayward) adopt the viewpoint of De Bary (pp. 126-134) and recognize two main "forms" of sclerenchyma; namely, "*stone cells*" which are short, more or less isodiametric elements and *fibers* which are prosenchymatous cells, often extremely long and with pointed or oblique ends. Despite the convenience of this morphological subdivision of sclerenchyma it seems hardly justified when the numerous structural and ontogenetic differences between so-called stone cells and fibers are clearly understood. For this reason, the term "sclerenchyma" appears vague and highly abstract and, as will appear in the resumé to follow, has been used to designate cell types which are definitely unrelated in wall structure and method of development (cf. Haberlandt, p. 721, note 92).

The expression "sclereide" was originally proposed by Tschirch (1885, p. 308) for the variously formed thick-walled cells which occur so commonly in the bark of woody seed plants, in the hard shell of fruits, and in seed coats. In contrast to typical "bast fibers," the thick walls of sclereides often appear yellow in color, are usually highly lignified and possess tubular pits which may branch in a complex fashion. On the basis of form and structure, Tschirch (1889, pp. 301-302) distinguished four principal types of sclereides which he named and described

as follows: (1) *Brachysclereides*, or "stone-cells" which are roughly isodiametric in form and which occur in the fleshy portion of the fruits of *Pyrus* and *Cydonia* and in the bark of numerous woody dicotyledons; (2) *Macrosclereides*, or "rod-cells" which are columnar in form and often constitute an outer continuous palisade-like layer in the coat of seeds, especially in the family Leguminosae. Here they are also referred to as "Malpighian Cells"; (3) *Osteosclereides*, or "bone-cells" which are likewise columnar in form but possess dilated or knob-like ends. Such cells occur within the palisade parenchyma in certain leaves (cf. Haberlandt, p. 160, Fig. 52); and (4) *Astrosclereides* or "branched sclereides," which are highly irregular in form and size with pointed "arms." Astrosclereides are well developed in the leaves of certain dicotyledons (e.g., *Thea*, *Camellia*) and also occur in the bark of *Abies* and *Larix* and in certain fruits (e.g., *Carya*).

The *ontogeny of sclereides* presents many distinctive and peculiar features. Aside from the macrosclereides, which are traceable in origin to the surface cells or "protoderm" of the integument of the ovule (cf. Zimmerman, 1936), sclereides usually develop by the "secondary sclerosis" of parenchymatous cells (cf. De Bary, pp. 539-544). This curious process involves the marked centripetal increase in thickness of the cell wall, the deposition of lignin within the cellulosic matrix and the production of the characteristic "pit-canals" or "ramiform pits." The physiological factors which induce these changes in a living parenchyma cell or cell group in the cortex or the bark of stems or roots are obscure. Tschirch (1889, p. 303) states that in woody dicotyledons, the process of secondary sclerosis occurs to such an extent that sclereides may eventually constitute the major portion of the bark. A further interesting aspect of the process of secondary sclerosis is exhibited by the development in many stems of the so-called "composite cylinder" formed of both brachysclereides and bast fibers. In this case, an originally continuous cylinder of bast fibers becomes ruptured at various points as a result of the increase in thickness of the stem. Neighboring parenchyma cells then intrude into the gaps, divide and eventually become transformed into sclereides thus "repairing"

the broken cylinder (for details cf. Tschirch, 1885, p. 323 et seq., and Haberlandt, p. 159). With respect to the ontogeny of osteosclereides and astrosclereides, little information appears to exist. In the husk of the fruit of hickory (*Carya*) a comparison of young and old material clearly suggests that the irregular astrosclereides arise much like brachysclereides from the secondary sclerosis of parenchymatous elements. But the details of the development of the huge astrosclereides occurring in the leaves of such plants as *Camellia* (Haberlandt, p. 162, Fig. 54) deserve careful investigation.

The statement is frequently made that stone-cells are devoid of a protoplast at maturity. This idea requires further proof because Alexandrov and Djaparidze (1927) contend that it is possible to demonstrate, by staining with safranin and methyl green, the presence of nuclei in the mature brachysclereides of the fruits of quince (*Cydonia*) and pear (*Pyrus*). These investigators further maintain that during the ripening of the fruit in *Cydonia*, the sclereides experience a process of "delignification" consisting in the reduction in thickness of the wall, the disappearance of lignin, and the obliteration of the ramiform pits. This reversible change suggests enzymatic activity on the part of the protoplasm within the stone cells. However, Crist and Batjer (1931) reached a different conclusion in their detailed study of the stone-cells of *Pyrus*. They state that the delignification reported by Alexandrov and Djaparidze for *Cydonia* does not occur in Kieffer and Bartlett pears . . . "without exception, the downward trend of the cellulose curve is strictly parallel to that of lignin and each one of the two is parallel to the ligno-cellulose trend." Further study is obviously needed to determine more precisely the chemical relations between the sclereides and the neighboring parenchyma tissue during fruit ripening.

From a functional standpoint, sclereides undoubtedly impart hardness to the organ in which they occur. Haberlandt (p. 158) states that brachysclereides "serve to increase the incompressibility of the bark; their action may be compared to that of the sand which a mason uses to increase the tenacity of his mortar, or to that of the powdered glass which is added to gutta-percha in order to render it less compressible." The functional signifi-

cance of the groups or "nests" of brachysclereides in fleshy fruits is less evident. It has been suggested that phylogenetically they may represent the remains of a former continuous shell of stone cells.

II. Material for the Study of Sclereides.—

1. *Brachysclereides or "stone-cells" in the fruit and fruit stalk of Pyrus.* Obtain a small fragment of the fruit of pear and mount it in water under a cover glass. Imbedded in the thin-walled parenchymatous tissue will be found small groups or "nests" of stone-cells which appear yellowish-brown in color. Examining one of these groups of stone-cells under high magnification note the form of the cells, their greatly thickened walls and the characteristic *ramiform pits*. After this preliminary examination, remove the cover-glass, add several large drops of a saturated solution of *phloroglucinol* [1] followed by a drop or two of hydrochloric acid. Note the brilliant red color assumed by the walls of the sclereides. Often this color change, which occurs when lignin is present in the walls, aids in the study of the branching and the relationship of the ramiform pits. Study carefully the form of the pits in both sectional as well as face view.

Secure a trans-section of the fruit stalk of *Pyrus* and treat it with phloroglucinol and hydrochloric acid as described above. Add a cover slip and examine the section under both high and low magnification. The edge of the section is formed of several layers of *cork* the innermost cells of which are in contact with the *phellogen* or cork cambium. Internal to the phellogen occurs the *cortex* which is composed of thick-walled parenchyma tissue in which are imbedded nests of *stone-cells*. Progressing inwards, there next occurs a "ring" of *fibrovascular bundles*. Each of these bundles consists of an external cap of *fibers* (which are usually less brilliantly stained than the stone-cells of the cortex), a strand of *phloem* and a strand of *xylem*. The *pith* of the fruit stalk is composed of both parenchyma as well as groups of stone cells.

2. *Astrosclereides*. Obtain a preparation of macerated "husk" of the fruit of *Carya* or *Juglans* and examine it under low mag-

[1] Cf. Appendix, p. 141.

nification. The two most abundant cell types are (1) *parenchyma cells* which vary from nearly isodiametric elements to irregular forms, and (2) *astrosclereides* which exhibit remarkable variation in the form and proportion of the "arms." The thick stratified wall and branched pits show very clearly in these sclereides. It is instructive to note the frequent similarity between certain of the parenchyma cells and the sclereides, indicating the origin of the latter from parenchyma by the process of secondary sclerosis which has been described in the Introduction of this exercise.

For comparative purposes, examine trans-sections of the petiole of the leaf of *Camellia* after staining them in phloroglucinol and hydrochloric acid. Study the sections under high magnification and note the elaborately branched areas of astrosclereides. Sections through the lamina of the leaf should also be stained and examined for the huge branched sclereides which occur in the midst of the mesophyll.

3. *Macrosclereides.* Obtained a small amount of macerated bean testa and examine it under low magnification noting the small, tightly packed groups of columnar macrosclereides. Note that the lumen of each macrosclereide is widest near the base of the cell, being reduced to a narrow, virtually occluded channel above. For illustrations of macrosclereides *in situ* in the testa of seeds of the Leguminosae refer to Eames and MacDaniels (p. 294, Fig. 134c), Hayward (p. 342, Fig. 174), and Netolitzky (1926, p. 159).

III. Suggested Drawings and Notes.

1. Prepare drawings to show the form and pit relationships of a small group of stone-cells in the fruit of the pear. How may the fact be explained that some of the ramiform pits or their branches fail to terminate at the "edge" of a stone-cell at a given level of focus?

2. Make a diagrammatic drawing of the trans-section of the fruit stalk of the pear, showing and labeling all the important tissues and regions. Summarize, in the form of notes, the positions and mechanical significance of the stone-cells and fibers in this organ.

3. Draw several types of astrosclereides from the macerated "husk" of the fruit of *Carya* or *Juglans*. For comparative purposes, draw a parenchyma cell resembling in its form one of these sclereides.

4. Prepare a diagrammatic drawing of the trans-section of the petiole of *Camellia*, showing the position of the astrosclereides with reference to other tissues. Draw a single astrosclereide, as seen under high magnification, from the petiole and from the lamina of the leaf.

5. Draw a few connected macrosclereides from the testa of the bean seed. Show carefully the wall structure and the form of the lumen.

REFERENCES

1. Alexandrov, W. G., and Djaparidze, L. I., Über das Entholzen und Verholzen der Zellhaut. Planta 4:467-475. 1927.
2. Crist, J. W. and Batjer, L. P., The Stone Cells of Pear Fruits, Especially the Kieffer Pear. Ag. Expt. Station Michigan State College. Tech. Bulletin 113. 1931.
3. De Bary, pp. 126-128.
4. Eames and MacDaniels, pp. 57-59.
5. Haberlandt, pp. 158-161.
6. Hayward, p. 25.
7. Netolitzky, F., Anatomie der Angiospermen Samen. Handbuch d. Pflanzenanatomie. X. 1926.
8. Tobler, F., Die mechanischen Elemente und das mechanische System. Handbuch d. Pflanzenanatomie. IV. 1939.
9. Tschirch, A., Beiträge zur Kenntniss des mechanischen Gewebesystems der Pflanzen. Jahrb. Wiss. Bot. 16:303-335. 1885.
10. ————————, Angewandte Pflanzenanatomie. Wien und Leipzig. 1889.
11. Zimmermann, K., Zur physiologischer Anatomie der Leguminosentesta. Landw. Versuchs-stat. 127:1-56. 1936.

Exercise IX

FIBERS

I. Introduction.—In the strict sense, the term "fiber" should be applied only to certain prosenchymatous cells found in the inner tissues or tissue systems of the plant body. Cells of this type are not to be confused with the so-called cotton "fibers" which morphologically represent epidermal hairs of the seed coat (cf. Anderson and Kerr, 1938). The term "fiber" is employed in the present book in the above restricted anatomical sense.

Fibers are the most important type of mechanical cell which occur in higher plants where their great tensile strength, flexibility and elasticity serve to enable plant organs successfully to withstand a variety of strains and tensions resulting from the action of gravity, wind, etc. (Cf. Haberlandt, pp. 161-164.) From a commercial standpoint, many plants are cultivated largely or exclusively for the fibers which they produce. Among the more prominent of such textile plants may be mentioned *Agave* sp., the source of "Sisal Hemp," *Musa textilis* from which "Manila Hemp" is derived, *Cannabis sativa* or the "true" hemp plant, and *Linum usitatissimum* which furnishes the commercial flax from which linen is derived. According to Hayward (p. 371), "there is evidence that flax was grown during the Stone Age" and that the annual form of *Linum usitatissimum* "has been grown in Mesopotamia for at least 4000 years."

Because of the considerable economic importance of fibers, a very extensive literature has developed. The limited scope of this book however precludes any effort to discuss in detail the many involved problems of wall structure and methods of development of fibers. Instead, a brief resumé is given now of the salient features of fibers which may serve as an introduction to

the subject. For students wishing additional technical information, reference should be made to the literature cited by Hayward under *Cannabis* (pp. 244-245) and *Linum* (pp. 409-410).

1. *Classification.* Fibers, either singly or more commonly in the form of strands or cylinders, are widely distributed in the plant body. In stems and roots, fibers are commonly found in the cortex, pericycle, phloem and xylem. In the leaves of many monocotyledons (e.g., *Musa*, palms, *Agave*, etc.), fibers are very prominently developed, occurring as strands or sheaths which accompany the vascular bundles; they may also appear independently of the vascular strands, either as distinct bundles or as massive hypodermal cylinders. (Cf. Haberlandt, pp. 168-184, and Meeuse, 1938.) From a *topographical standpoint*, two principal "types" of fibers may be recognized, at least in stems and roots which experience secondary growth in thickness, viz.: (1) *Bast* or extracambial fibers, and (2) *Wood fibers* or intracambial fibers. As Haberlandt (p. 155) has clearly emphasized, such a distinction is quite arbitrary since bast fibers, as a class, cannot be distinguished on a structural basis from wood fibers. Eames and MacDaniels (p. 57) and Hayward (p. 23) suggest that fiber types should be more specifically designated according to the tissue or tissue region in which they occur, e.g., cortical fibers, pericyclic fibers, phloem fibers, etc. A classification of this kind, however, necessarily depends upon accurate information on the origin and development of the fibers in each particular case. This is very clearly shown by the recent investigations of Esau (1938b, pp. 367-369) on the origin and development of the fibers in the stem of tobacco. In this plant, Esau interprets the fibers morphologically as part of the primary phloem rather than as "pericyclic fibers," as has been done by certain workers. It is evident from Esau's discussion of the literature that there is a great need for a complete re-examination of the concept of "pericycle" from an histogenetic point of view. Under such circumstances, the classifications of fibers into "bast fibers" and "wood fibers" will be followed in this book largely for the sake of simplicity and convenience.

2. *Form and length of fibers.* Fibers are classical examples of typical prosenchymatous cells. The ends are either acute or

acuminate or, as in certain bast fibers, variously "lobed" or "branched" (Mansfield, p. 22, Fig. 2). In short fibers, the ratio of the diameter of the cell to its length may average 1:10 to 1:20 while in extreme cases (e.g., in the Urticaceae) the ratio may reach or exceed 1:4000. These data, taken from De Bary (p. 13), emphasize the fact that certain bast fibers may represent the largest of all cells in higher plants. According to Hayward's (p. 241) discussion of the literature, hemp (*Cannabis sativa*) fibers vary in length from 1-10 cm. In flax (*Linum*) the length of the fibers likewise varies from 2.5 cm. to as high as 12 cm. Apparently the longest bast fibers which have been accurately measured occur in the stem of *Bochmeria nivea*, a member of the Urticaceae. In this species, Aldaba (1927) succeeded, by means of a special maceration technique, in isolating individual fibers, the five longest of which measured "respectively 400, 500, 520, 540, and 550 mm."

3. *Structure and chemistry of the cell wall.* Mature fibers characteristically possess a well-defined secondary wall which is often so thick that the cell lumen may be almost or entirely occluded at various points. The thick secondary wall exhibits typically slit-like vestigial pits which in bast fibers are disposed obliquely in a left-handed spiral series. Haberlandt (p. 154) contends that this arrangement of pits indicates a "corresponding arrangement of the micellar rows" and that "an obliquely pitted bast-fiber may therefore be regarded as an aggregate of exceedingly numerous and delicate fibrillae twisted together into a spiral coil of many turns which surrounds a longitudinal canal consisting of the cell cavity." Because of the great economic importance of certain fibers, many studies have been devoted to the chemical composition of their walls. Hayward (p. 23), while admitting that the degree of lignification of the cell wall may vary even within the same zone of fibers, distinguishes between (1) *non-sclerotic* fibers, which occur commonly in the pericycle of stems and which possess secondary walls with a relatively high proportion of cellulose (e.g., *Linum*), and (2) *sclerenchymatous fibers*, which are part of the xylem and which exhibit highly lignified secondary walls. According to Hayward, lignification tends to render fibers rather brittle while the high cellulose content of

the walls of certain bast fibers is related to the greater tensile strength and durability of such elements.

4. *Ontogeny of fibers.* Regardless of their location in the plant, fibers arise from *initial cells* which are very short as compared with the length of the mature element. An impressive example of this fact is furnished by Aldaba's (1927) work on fiber development in *Bochmeria.* In this plant, the fiber initials "are approximately 20 microns in length" and "the increase in the longitudinal dimension of the longer bast fibers is of the order of 2,500,000 per cent, but the process of elongation is gradual and extends over a number of months." The mechanics of the process of elongation in fibers and the accompanying development of a thick secondary wall has attracted much attention as well as speculation. The investigations of Aldaba (1927) and Anderson (1927) on flax fibers have revealed many peculiarities but our knowledge of fiber development in other forms is still meagre. It is apparent that in certain bast fibers, the upper end of the element remains delicate and active during the phase of cell elongation. Whether the necessary adjustment between such greatly extending cells and their neighbors is achieved by "sliding" growth or by "differential" growth, however, is not clear. (Cf. Anderson 1927, Meeuse 1938 and Hayward 1938, pp. 395-400.) The behavior of the protoplast during the growth and differentiation of certain types of fibers offers a number of points of interest. In a recent study, Esau (1938a) has shown that during the elongation of the primary phloem fibers in tobacco, the protoplasts become multinucleate as a result of repeated mitotic divisions of the nuclei. Cell plates, however, do not form at the end of the successive nuclear divisions and "the spindle fibers are less persistent than in ordinary division figures." At the final stages in fiber ontogeny, usually after secondary walls have developed, the nuclei appear to fuse or clump and in nearly mature fibers "the nuclear material frequently occurs as one large degenerating mass." The physiological significance of this multinucleate condition in young phloem fibers is quite obscure. Haberlandt (p. 154) who has observed a multinucleate protoplast in the bast fibers of *Linum* and certain members of the Leguminosae maintains that "the presence of several nuclei appears

advantageous when the very considerable length of many bast-cells and their active growth in length and thickness are taken into account." In certain types of wood fibers, however, mitosis is followed by cytokinesis, resulting in a chambered or *septate fiber*. This condition has been observed and described by Vestal and Vestal (1940) in a recent study of the septate fiber-tracheids of *Hypericum Androsaemum*. In this species, the fiber-tracheid retains its protoplast after the thick secondary wall has been laid down. Mitosis may then occur in such a cell, the division figure being oriented parallel to the long axis of the cell. Cell plate formation then occurs in the normal manner and a thin transverse septum is formed across the lumen, intersecting the inner edge of the secondary wall of the "mother cell." Because of the delicacy of this septum it was not possible to determine whether it is "formed only of intercellular cement substance or whether it consists of the intercellular substance and two adjacent primary walls."

II. Material for the Study of Fibers.—

1. *Bast fibers.* Examine, under low and high magnification, macerated bark of the twig of the basswood or linden tree (*Tilia* sp.). The numerous prosenchymatous cells present are bast fibers from the phloem region. Select an unbroken fiber and study carefully its form and wall structure. Note especially the channel-like lumen and the small *vestigial pits*. According to Eames and MacDaniels (p. 57), the pits in bast fibers represent modified simple pits. To appreciate fully the arrangement and mechanical significance of the fibers in the phloem of *Tilia*, strip off a small portion of the bark from a twig or young branch and scrape off the outer tissues (i.e. epidermis, periderm and cortex) with a scalpel. Then mount the fibrous tissue which has been exposed in water and examine it under low magnification, noting the *closely-joined strands* of grayish-white bast fibers. In order to determine the degree of lignification of the secondary walls, treat separate portions of the fibrous network with (1) IKI and sulphuric acid,[1] and (2) phloroglucinol and hydrochloric acid.[2]

[1] Cf. Appendix, p. 141.
[2] Cf. Appendix, p. 141.

If time permits, make similar studies and microchemical tests of the bast fibers of various economically important textile plants, such as "hemp" (*Agave* sp. and *Cannabis sativa*) and flax (*Linum usitatissimum*).

2. *Wood fibers.*

(*a*) *Libriform fibers.* The fibers present in the secondary xylem of woody dicotyledons often show massively thickened secondary walls provided with scattered and rather small vestigial pits. In such cells, which were termed "libriform" because of their structural resemblance to phloem fibers, the lumen varies in width and may be entirely occluded at certain points (cf. Eames and MacDaniels, p. 63, Fig. 34, e, f, g). Study the form and structure of the libriform fibers as shown in macerated wood of oak (*Quercus* sp.).

(*b*) *Septate fibers.* This type of wood fiber is characterized by the subdivision of the lumen into a series of compartments which are separated from each other by transverse walls or septa. The work of Vestal and Vestal (1940) discussed in the Introduction of this exercise has shown that in *Hypericum*, the septa of the fiber-tracheids arise, *after* the formation of the lateral secondary wall, as a result of repeated mitoses accompanied by cytokinesis. Doubtless the septate fibers in other genera of the angiosperms pursue a similar ontogeny. Secure a small amount of macerated xylem from the stem of the grape-vine (*Vitis* sp.) and study under high magnification the form and structure of the numerous septate fibers. Note that the septa in these cells extend to the inner edge of the secondary wall but are independent of the compound middle lamella of the "mother cell." If the septa are examined with the aid of an oil-immersion lens, it is apparent that they have a laminated as well as a "pitted" structure.

III. Suggested Drawings and Notes.—

1. Prepare careful drawings to illustrate the form, character of the lumen and the type and distribution of pits in the bast fiber of *Tilia* and the libriform fiber of *Quercus*. Label all important structures.

2. Prepare a diagram to illustrate the arrangement of the strands of bast fibers in the stem of *Tilia*.

3. Draw a single septate fiber from the secondary xylem of *Vitis* showing its form, pitting and the position and structure of the septa.

4. Prepare a brief resumé of the commercial process of "retting" fibers in hemp or flax (for literature and information cf. Hayward, pp. 242, 244-245 and 409-410, and Anderson, 1927).

REFERENCES

1. Aldaba, V. C., The Structure and Development of the Cell Wall in Plants, I. Bast fibers of *Boehmeria* and *Linum*. Amer. Jour. Bot. 14:16-24. 1927.
2. Anderson, D. B., A Microchemical Study of the Structure and Development of Flax Fibers. Amer. Jour. Bot. 14:187-211. 1927.
3. ————, —— and Kerr, T., Growth and Structure of Cotton Fiber. Indus. and Eng. Chemistry 30:48-54. 1938.
4. De Bary, pp. 128-134.
5. Eames and MacDaniels, pp. 56-57, 62-64 and p. 75.
6. Esau, K., The Multinucleate Condition in Fibers of Tobacco. Hilgardia 11:427-434. 1938a.
7. ————, ——, Ontogeny and Structure of the Phloem of Tobacco. *Ibid.*, 11:343-424. 1938b.
8. Haberlandt, pp. 152-155.
9. Hayward, pp. 23-25.
10. Mansfield, pp. 89-106.
11. Meeuse, A. D. J., Development and Growth of the Sclerenchyma Fibers and Some Remarks on the Development of the Tracheids in Some Monocotyledons. Rec. trav. bot. neerlandais 35: 288-321. 1938.
12. Vestal, P. A. and Vestal, M. R., The Formation of Septa in the Fiber-tracheids of *Hypericum Androsaemum* L. Harvard Bot. Mus. Leaflets 8:169-180. 1940.

Exercise X

TRACHEARY ELEMENTS

I. Introduction.—One of the most characteristic features of the sporophyte of the "Tracheophyta" is the presence of a well-defined *conductive* or vascular system. This system is histologically complex in that its two component "tissues," i.e., *phloem* and *xylem*, are formed of a variety of cell types differing in their arrangement, form, protoplasts and wall structure. While the functions of storage and mechanical support are performed to varying degrees by both the phloem and the xylem, these "complex tissues" are concerned first of all in the *trans-location* of water and solutes between root and shoot. This conductive function is possible not only because of the structural characteristics of the cells themselves but also because the vascular tissues in the root, stem and leaves are interconnected and form a continuous system. From this standpoint, the vascular system may be visualized as an internal "skeletal" framework to which new increments are added during growth by the activity of the apical meristems and, in plants with secondary growth, the vascular cambium. This exercise is devoted to an introductory study of the xylem, with particular emphasis upon the structure and development of its definitive tracheary elements, viz.: the *tracheid* and the *vessel*. In the exercise dealing with the anatomy of the root, stem and leaf, additional information regarding xylem and directions for studying it will be given.

1. *Structure and morphology of tracheary elements.* The expression "tracheary elements" is used in this book to designate collectively the tracheid and the vessel element which represent the two chief types of water-conducting cells present in the xylem of vascular plants. The following characteristics are common to both tracheids and vessel elements, viz.: (1) they are typically *prosenchymatous* in form with oblique or pointed ends. An exception to this is furnished by the cylindrical form of vessel

elements in certain angiosperms. (2) The secondary wall of mature tracheary elements consists of lignified cellulose and is deposited as rings, spiral bands, bars, a reticulum or as pitted layers upon the thin primary wall; (3) at maturity, tracheary elements lack a protoplast and the lumen is occupied by gas or fluids. The principal *distinction* between the two types of tracheary elements consists in the fact that the tracheid is an *imperforate* cell with a continuous primary wall, while a vessel element is provided with distinct openings or *perforations* which are usually located in the end-walls of the cell. When longisections of the xylem are examined, it is evident that intercommunication between adjacent tracheids is possible by means of the bordered pit-pairs on their *lateral walls*. In contrast, vessel elements occur in more or less distinct vertical series in which the perforations of adjacent elements exactly coincide. Thus, collectively regarded, a series of vessel elements constitutes an open "pipe-like" structure which is termed a *vessel*. The literature devoted to the structure and pitting of tracheary elements is so extensive that it will only be possible to outline below some of the most salient features of tracheids and vessel elements.

(a) *The tracheid*. From a phylogenetic standpoint, the tracheid is usually regarded as the "fundamental" cell type in the xylem of vascular plants. According to Eames and MacDaniels (p. 62), "tracheids alone probably made up the xylem of very ancient plants." Among living plants, tracheids constitute the only type of tracheary element in the xylem of most lower vascular plants and, except for the Gnetales, are the dominant cell type in the wood of gymnosperms. Tracheids are also characteristic of angiospermous xylem where together with vessel elements, fibers and parenchyma they contribute to produce the great histological complexity typical of the wood in this group. Structurally, the tracheid is an elongate cell, the secondary wall of which is laid down in a variety of patterns. In *primary xylem*, i.e., the xylem which develops first in the ontogeny of the root, stem and leaf, the secondary wall has the form of rings, spiral bands, bars, a network or else is provided with distinct pits. A more detailed discussion of the "fibrous" types of secondary wall-thickening in the tracheary elements of primary

xylem will be given later in this Introduction. In *secondary xylem*, i.e., the wood produced by the vascular cambium, the walls of the tracheid are provided either with transverse, slit-like *scalariform bordered pits* (as in ferns, and club mosses) or with *circular or oval bordered pits* (as in most gymnosperms and angiosperms). The type and arrangement of the pits of tracheids seem to be determined in part by the nature of the cell or cells bordering the tracheid. Thus when two tracheids are in contact, bordered pit-pairs occur while, if a wood-parenchyma or wood-ray cell crosses the tracheid, half-bordered pit-pairs are developed. Frost (1929) in a study of angiosperm xylem, however, has called attention to the fact that the type of pitting in a given tracheary element depends to a large extent upon the degree of phylogenetic specialization of the cell itself rather than upon the type of neighboring cells. Frost finds that the pit-pairs between tracheary elements and parenchyma cells may be either bordered, half-bordered, or simple, according to the species and that this situation constitutes a reliable criterion for distinguishing the xylem of various plants. It seems clear that the whole question of tracheary pitting demands more study both from a comparative as well as an ontogenetic point of view. Indeed, considerable diversity of opinion prevails as to the nature of the most primitive type of pitting in seed-plants (cf. Jeffrey Ch. IV and VII, Brown 1918 and Bliss 1921).

(*b*) *The vessel element.* This type of tracheary element is generally interpreted as having evolved, phylogenetically, from some primitive type of tracheid. In the genus *Ephedra* for example, the sloping end walls of certain of the tracheary elements are provided with circular bordered pits, the membranes of some of which disappear during ontogeny. Cells of this type may typify one of the ways in which the *perforations*, distinctive of vessel elements, may have orginated (cf. Jeffrey, pp. 94-95, Figs. 72-73). Further evidence of the derived nature of vessel elements is afforded by their distribution in extinct and living vascular plants. According to Jeffrey (p. 93), vessels are absent from the secondary wood of Paleozoic cryptogams. Among existing lower vascular plants, vessels are known to occur in certain species of *Selaginella* (Duerden 1934) and in two species of *Pteridium*

(Bliss 1939). Save for these examples, and the Gnetales, vessel elements seem to be restricted in their occurrence to the xylem of angiosperms. The recent survey of Cheadle (1939) has brought out the interesting fact that while vessels occur consistently in the roots of monocotyledons, this type of tracheary element is comparatively infrequent in the xylem of the stems and leaves of this class of the angiosperms. The significance of this condition remains to be explained. In the dicotyledons, vessels apparently are of widespread occurrence in both primary and secondary xylem, and have only been reported absent in *Drimys*, *Trochodendron*, *Tetracentron* (cf. Bailey and Thompson 1918) and certain members of the Crassulaceae and Cactaceae.

Two principal types of perforations occur in vessel elements, viz.: (1) the *simple perforation*, which appears as a single large oval or circular hole in each of the end-walls of the cell, and which is interpreted as the more advanced condition, and (2) the *scalariform perforation* or perhaps more accurately the *scalariform perforation plate*, which appears as a series of elongated parallel openings separated by transverse bars, and which is usually regarded as the more primitive type of perforation. Simple perforations occur in vessel elements with either sloping or transverse end-walls while scalariform perforations are typical of elements with oblique or strongly-inclined end-walls (cf. Eames and MacDaniels p. 65, Fig. 35). According to the investigations of Cheadle (1939), in the majority of monocotyledons examined the vessel elements possess the scalariform type of perforation plate. A full discussion of the possible evolutionary history of the perforation-plate in vessel elements of the seed plants is given by Jeffrey (pp. 92-102) and Bliss (1921).

2. *Ontogeny of tracheary elements.*

(*a*) *The formation of perforations in vessel elements.* Recent studies of this problem have centered about two important questions, viz.: (1) the exact period or *time* in vessel differentiation when the dissolution of the end-walls occurs, and (2) the *physical and chemical nature of the portion of the end-wall* which becomes perforated. According to Eames and MacDaniels (p. 151), the perforation of the transverse end-walls of the vessel elements in the secondary xylem of the black locust (*Robinia*

Pseudo-Acacia) occurs *after* the cells have reached their full size and have developed secondary walls over all portions of the element. Perforation in this species therefore involves the dissolution of a portion of *both* the secondary as well as the primary layers from the central region of the end-walls. Esau (1936), however, in her study of vessel development in the primary xylem of celery, found that the secondary thickening, in the form of spiral bands, is restricted to the lateral walls and to a thin peripheral region of the transverse end-walls. The perforation in this case involves the breakdown of a distinct central region of the end wall which is interpreted as primary in nature. This area in section view appears as a lenticular thickening and is "similar to the torus thickening in bordered pits." In a later publication, Esau and Hewitt (1940) investigated the nature of the end-walls and the development of perforations in the vessel elements of *Cucurbita pepo*, *Zea mays*, *Nicotiana tabacum*, *Daucus carota* and *Beta vulgaris*. The end-walls of *Beta*, *Daucus* and *Nicotiana* agree with those in celery in possessing a conspicuous lens-shaped thickening which breaks down to form the simple perforation during the final stages of vessel development, *after* secondary walls have been formed. Perforation of the vessel elements in *Cucurbita* occurs at a similar period in ontogeny but in this form the portion of the end-wall to be dissolved is not lens-shaped in sectional view. As a result of careful microchemical and optical tests of the end-walls, Esau and Hewitt conclude that "two superimposed vessel elements are separated from each other by two cellulose layers—the two primary walls—cemented together by isotropic intercellular substance."

(*b*) *The nature and origin of "fibrous" thickenings in primary xylem tracheae.* The secondary wall of the tracheary elements in primary xylem is deposited upon the delicate primary wall in a number of well-defined patterns. In the *protoxylem* or first-formed portion of the primary xylem, the secondary wall appears in the form of separate rings (*annular elements*), one or more spiral bands (*spiral elements*) or as transverse, interconnected bars (*scalariform elements*). These distinctive types of tracheary elements usually originate successively in provascular tissue ("procambium") in the order named above, although the

proportion of each type varies within wide limits in different organs and different plants. The assumption is frequently made that the significance of these various wall-patterns in protoxylem tracheae is to permit the elements to "accommodate themselves" to stretching. In the *metaxylem* or last-formed portion of the *primary xylem*, there is a marked increase in the relative extent of secondary wall deposition, and elements with net-like thickenings (*reticulate elements*) and with pitted walls (*pitted elements*) are formed. A sharp transition does not exist, however, between these varied wall-patterns and consequently the limits between protoxylem and metaxylem can only be rather arbitrarily established. Indeed, it is common to find tracheary elements with several types of intergrading wall-patterns. Such transitional types were early recognized and termed "vasa mixta." (Cf. De Bary, p. 156.)

Apparently very little intensive study has been devoted to the origin and mode of development of the fibrous type of secondary wall thickening in primary xylem tracheae. Stover (1924) contends that in *Calamovilfa* "the annular and spiral thickenings are the direct *result* of elongation." He finds that the "first thickening is laid down in the pitted form" and that "the division and enlargement of the surrounding cells tears apart this wall thickening and the cell becomes annular, spiral or reticulate, depending upon the amount of stretching." This interesting mechanical interpretation, however, could not be confirmed by the work of Barkley (1927) on the differentiation of tracheary elements in *Trichosanthes*. According to Barkley, the future pattern of the secondary wall is determined early in ontogeny by a peculiar distribution of vacuoles in the peripheral cytoplasm of the procambial initial. She states that "the spiral vessel of the protoxylem in its early stages has bands of peripheral cytoplasm which precede the spiral markings and have the same arrangement, and become the basis of the lignified spiral. The position of the cytoplasmic bands is determined by rows of vacuoles in the cytoplasm immediately preceding and during the formation of the cytoplasmic bands." In a similar way, the annular and reticulate types of thickenings are predetermined by the pattern of vacuolation in the cytoplasm. It is evident, however, that a

careful cytological study of this problem from an extensive as well as an intensive standpoint is needed in advance of any generalizations. With the aid of recent improvements in plant microtechnique, it should be possible to investigate successfully the precise relationships between the cytoplasm and the development of specific wall-patterns in tracheary elements.

3. *The distinction between primary and secondary vascular tissues.* In practice, great difficulty is experienced in attempting to distinguish the boundaries between the primary and secondary vascular tissues especially in leaves and young stems. Protoxylem, because of the definitive characters of the secondary wall patterns, is usually readily demarcated but the limits between metaphloem and secondary phloem on the one hand, and metaxylem and secondary xylem on the other hand, are often difficult or indeed impossible to draw on the basis of adult structure. One of the criteria often employed is based on the idea that the conducting elements of secondary vascular tissues, since they originate from periclinal derivatives of the cambium, are arranged in more or less regular radial rows in contrast to their irregular arrangement in primary phloem and primary xylem. In a recent review of this whole problem, Esau (1938, pp. 356-361) has shown, however, that according to many ontogenetic studies, the first-formed or "primary" vascular tissues "may be arranged in an orderly manner." A good illustration is furnished by the radial alignment of both "primary" and "secondary" tracheary elements in the vascular bundle of *Trifolium* (Eames and MacDaniels, p. 255, Fig. 117A). Evidently then, the method of arrangement of tracheary cells does not provide a consistent basis for demarcating primary and secondary xylem. However, when certain differences are analysed between the so-called procambium, which produces the primary vascular tissue and the "cambium" which forms secondary vascular tissues, it appears that the distinction between the two tissues may have some justification ontogenetically.

According to Esau, four significant differences can be recognized, viz.: (1) in organs with well-defined secondary growth, two kinds of initials, the *ray* and *fusiform* types, are characteristic of the cambium. These initials produce respectively the

vascular rays, and the sieve-tubes, vertical parenchyma, fibers and tracheary elements. Usually procambial cells are very much alike in form and structure. (2) As seen in trans-section, procambial cells tend to be polygonal in form in contrast to the rectangular or "box-like" shape of cambial cells. (3) The maturation, especially of tracheary elements, from the cambium is more abrupt than is the case in the procambium, and (4) the radial walls of cambial cells are often noticeably thicker than the tangential walls, a distinction which is not apparently characteristic of procambial cells. In view of the above differences and because of the exploratory state of the problem, the terms "primary" and "secondary" will be utilized in this book as convenient designations for the vascular tissues derived respectively from the procambium and the cambium. But the author is in full agreement with Esau's statement that procambium and cambium are to be regarded "not as two distinct meristems, but as two developmental stages of the vascular meristem."

II. Material for the Study of Tracheary Elements in Primary Xylem.—

1. *Transverse and longi-sections of the hypocotyl of bean seedlings.* Treat the sections with phloroglucinol and hydrochloric acid,[1] mount in water and examine under low and high magnification. Note carefully the irregular arrangement of the tracheary elements in the differentiating primary xylem as shown in the trans-sections. The longi-sections, if cut in the radial plane with respect to the primary xylem, will show the order of appearance and the types of secondary wall-patterns in the tracheary elements of the protoxylem and metaxylem.

2. *Prepared and stained transverse and longi-sections of the stem of Trifolium.* Examine the trans-sections noting particularly the regular arrangement of the tracheary elements in the primary xylem of the collateral vascular bundles. A study of the longi-sections will reveal not only the various types of wall-patterns in the successive tracheary elements, but will also show the effects of stretching on the primary and secondary walls of the annular and spiral elements of the protoxylem.

[1] Cf. Appendix, p. 141.

3. *Macerated primary xylem of the hypocotyl of bean and the stem of Trifolium.* Obtain small quantities of the macerated tissue and study the form and wall-patterns of the various types of isolated tracheary elements. Although many of the elements may be broken or injured in the process of maceration, it is possible to find intact tracheids or vessel elements. Note especially the intergradations in certain elements between several different types of secondary wall thickening. It is also instructive to contrast the appearance of the rings and spiral bands in short and elongated protoxylem elements.

4. *Macerated primary xylem of the rhizome of the bracken fern (Pteridium latiusculum).* Obtain a small amount of macerated primary xylem of *Pteridium*, mount it in water and add a cover-glass. The individual tracheary elements are large cells, clearly visible to the naked eye, and vary in form from broadly-fusiform or obovate to narrowly-acuminate. Examine these cells under low and high magnification, noting that one or both end-walls are oblique with reference to the lateral walls. The latter are provided with vertical series of typical *scalariform bordered pits*. The presence of well-defined *scalariform perforation plates* on the sloping end-walls of certain of these cells indicates that they represent vessel elements. According to the recent work of Bliss (1939, p. 620) "there are many cells that may be interpreted as transitional between the tracheid and the vessel element."

III. Material for the Study of Tracheary Elements in Secondary Xylem.—

1. *Tracheids of gymnosperms.* Obtain a small quantity of macerated wood of *Pinus* and study the form and pitting of the tracheids. Three types of elements are present, viz.: (1) tracheids from the "spring wood," characterized by their relatively wide lumina and by the restriction of pits to the radial walls; (2) tracheids from the "summer wood," distinguished by their much narrower lumina and by having the pits confined to the tangential walls, and (3) fiber-tracheids which in their thickened walls and reduced pits are intermediate in character between "typical" fibers and tracheids. Note, especially in the spring tracheids, that at certain points in the cell there occur groups of large, in-

distinctly bordered pits which mark the point of contact between the tracheid and the living cells of a wood-ray. As seen in face view, the large circular bordered pits of a spring tracheid are separated from one another by bars of wall substance (cf. Eames and MacDaniels, p. 29, Fig. 17A). These wall-sculpterings are termed "Bars of Sanio" and have occasioned much speculation as to their significance. The Committee on Nomenclature of the International Association of Wood Anatomists, however, suggests that the term "Bars of Sanio" should be replaced by the term "crassulae" which are defined as "thicker portions of the intercellular layer and primary walls between primary pit fields." To appreciate fully the form and distribution of pits in conifer tracheids, a study should also be made of stained transverse, radial and tangential sections of pine wood.

2. *Tracheids of angiosperms.* Mount a small quantity of macerated oak wood in water and study carefully the form and pitting of the tracheids. These cells are distinguished from the very abundant wood fibers by their somewhat shorter length, wider lumina and more obviously bordered pits. Many of the tracheids will appear very irregular in contour with forked or lobed ends. (Cf. Eames and MacDaniels, p. 60, Fig. 33D). Under high magnification, note that the bordered pits are oval in form, often crowded and somewhat smaller than the larger circular bordered pits characteristic of the spring tracheids of *Pinus*.

3. *Vessel elements with scalariform perforations.* Macerated wood of birch (*Betula*) and of the tulip-tree (*Liriodendron*) will provide instructive examples of this primitive type of vessel element. Note the variation in the number and relative width of the slit-like openings in the oblique perforation plates of these two plants, and the variety in type and arrangement of pits on the lateral walls.

4. *Vessel elements with simple perforations.* As stated in the Introduction of this exercise, the simple type of perforation may occur in vessel elements with either oblique or more or less transverse end-walls. The first condition is shown by certain of the vessel elements in macerated oak wood. Note in this material that the pointed ends of the vessel element extend beyond each of the

large, oval terminal perforations. As in the vessel elements of *Betula* and *Liriodendron*, various types and patterns of pits are characteristic of the lateral walls. A study of the short cylindrical type of vessel element, with greatly-enlarged simple perforations in the transverse end-walls, may be made with the aid of stem-sections and macerated xylem of the pumpkin (*Cucurbita*). As seen in macerated tissue, the individual vessel elements are somewhat drum-shaped with densely-pitted lateral walls. The enormous diameter of these vessel elements and their union to produce vessels may readily be studied in hand-sections stained with phloroglucinol and hydrochloric acid. For a description and illustrations of the development and structure of vessels in *Cucurbita* reference should be made to the work of Esau and Hewitt (1940).

5. *The cellular composition of paper.* A large proportion of paper is obtained from the wood of certain gymnosperms (e.g., *Abies*, *Picea*) and angiosperms (e.g., *Populus*, *Betula*). The first stage in the manufacture of paper from wood consists in the mechanical and chemical maceration of the xylem which results in the partial dissociation of its component cells. This macerated xylem is commercially known as *wood pulp* and after being bleached, colored or "sized," according to the required use, is compressed under great pressure into paper sheets (cf. Kellog, 1923 for further details). It is interesting to note, however, that despite the drastic treatments involved in the production of wood pulp, many of the tracheary elements are well preserved and their structure and pitting is recognizable if small pieces of soaked paper are carefully teased apart in water and examined under the microscope. Make a study of the various types of cells found in newspaper, blotting-paper and some cheap grade of writing-paper.

IV. Suggested Drawings and Notes.—

1. Prepare drawings to show the shape, structure, and arrangement of the primary xylem tracheae in the vascular bundle of the bean hypocotyl or the stem of *Trifolium*, as seen in trans- and longi-sectional view. These drawings should be supplemented by showing, on a large scale, the details of small portions of the secondary wall-patterns of *isolated elements* of the proto-

xylem and metaxylem drawn from macerated primary xylem of the hypocotyl of the bean seedling.

2. Outline, on a large scale, the form of a single vessel element from the macerated primary xylem of *Pteridium*, indicating by horizontal lines the pattern of *scalariform bordered pits* on the lateral walls. Draw, as seen under high magnification, a scalariform perforation plate of one of these tracheary elements.

3. Prepare drawings of spring and summer tracheids of macerated pine wood, showing the type and arrangement of the pits as seen in face and sectional view. For comparison, draw a single tracheid from macerated oak wood.

4. Make drawings of vessel elements of macerated wood of *Liriodendron* (or *Betula*), *Quercus* and *Cucurbita*, to illustrate differences in the form of the elements and the type of perforation. Fill in the pits on a small portion of the lateral wall of each vessel element.

5. Summarize, in tabular form, the various types of cells observed in the specimens of paper studied. Indicate whether differences in the kinds of tracheary elements observed can be used to deduce the source of the paper in each case, i.e., from conifer or angiosperm wood.

6. Prepare a resumé, based upon the conclusions of Jeffrey, Brown (1918) and Bliss (1921) of the evolutionary development of the vessel in seed plants.

REFERENCES

1. Bailey, I. W., and Thompson, W. P., Additional Notes upon the Angiosperms, *Tetracentron*, *Trochodendron* and *Drimys* in which Vessels Are Absent from the Wood. Ann. Bot. 32:503-512. 1918.
2. Barkley, G., Differentiation of Vascular Bundle of *Trichosanthes anguina*. Bot. Gaz. 83:173-184. 1927.
3. Bliss, M. C., The Vessel in Seed Plants. Bot. Gaz. 71:314-326. 1921.
4. ————, ——, The Tracheal Elements in the Ferns. Am. Jour. Bot. 26:620-624. 1939.
5. Brown, F. B. H., Scalariform Pitting a Primitive Feature in Angiospermous Secondary Wood. Science 48:16-18. 1918.
6. Cheadle, V. I., The Occurrence of Vessels in the Monocotyledonae. Amer. Jour. Bot. 26. Supplement p. 9s. 1939.

7. De Bary, pp. 155-171.
8. Duerden, H., On the Occurrence of Vessels in *Selaginella*. Ann. Bot. 48:459-465. 1934.
9. Eames and MacDaniels, pp. 59-62, 64-67, 86-97, 150-153, 162-188.
10. Esau, K., Vessel Development in Celery. Hilgardia 10:479-488. 1936.
11. ————, ——, Ontogeny and Structure of the Phloem of Tobacco. Hilgardia 11:343-424. 1938.
12. Esau, K., and Hewitt, W. B., Structure of End Walls in Differentiating Vessels. Hilgardia 13:229-244. 1940.
13. Frost, F. H., Histology of the Wood of Angiosperms, I. The nature of the pitting between tracheary and parenchymatous elements. Bull. Torr. Bot. Club 56:259-264. 1929.
14. Haberlandt, pp. 302-325.
15. Jeffrey, Ch. IV and VII.
16. Kellog, R. S., Pulpwood and Woodpulp in North America. N. Y., 1923.
17. Stover, E. L., The Vascular Anatomy of *Calamovilfa longifolia*. Ohio Jour. Sci. 24:169-179. 1924.

Exercise XI

SIEVE-TUBE ELEMENTS

I. Introduction.—The phloem of vascular plants, like the xylem, is a "complex tissue" which may consist of four or five different types of cells. Nevertheless, the phloem is morphologically well defined by the consistent presence of a highly specialized kind of cell known as a *sieve-tube element* or *sieve-cell.* This definitive cell type lacks a nucleus at maturity and in addition possesses other structural and physiological properties which are apparently unique. In many angiosperms, the sieve-tube elements are clearly arranged in definite vertical series, to each of which the collective term "*sieve-tube*" may be applied. The term sieve-tube has also been given to the individual enucleate cells typical of the phloem in gymnosperms. Such cells, however, are not arranged in linear series. Abbe and Crafts (1939) refer to these structures in conifers as "sieve elements." As Esau (1939) has pointed out in a thorough review of the literature on phloem structure, the word "sieve-tube in its original meaning had reference to a series of superposed cells with transverse or somewhat inclined end walls bearing sieve-plates." Under such circumstances, the term "sieve-tube" will be reserved in this book for definable vertical cell-series and the term "sieve-tube element" used for the individual members of such a series. Similar cells of the phloem of gymnosperms, not arranged in vertical superposed series, will be designated as "sieve-cells."

In angiosperms, the sieve-tube elements are usually accompanied on one of their lateral walls by small prismatic or tubular cells. These cells, which are intimately connected with the sieve-tubes, are termed *companion cells* and differ from the sieve-tube elements in possessing nuclei and in lacking definite sieve plates. Companion cells are to be regarded as sister cells of the sieve-tube elements since both types of cells originate by the division of a common mother cell (cf. Esau, 1939, pp. 409-410). Sieve-

cells in the gymnosperms lack companion cells in the above sense, but specialized "*albuminous cells*" occur and have been regarded as equivalent to the companion cells in angiosperms. However, Abbe and Crafts (1939, p. 710) have questioned this analogy since the albuminous cells "do not arise from a common fusiform initial with the sieve-tube but from separate ray initials." The *primary phloem* is usually relatively simple in structure, consisting of sieve-tubes, companion cells and phloem parenchyma as in *Cucurbita* or only the first two types of cells may be present (e.g., *Zea Mays*). But *secondary phloem* may be very complex because of the presence of fibers, stone cells, vertical phloem parenchyma and rays in addition to sieve-tubes and companion cells. (Cf. Eames and MacDaniels, Ch. VIII, and Esau, 1939, pp. 411-413.)

Aside from the secondary functions of food storage and support, the chief physiological role of phloem is the *conduction of various organic solutes*. Experimental studies on translocation (cf. Crafts, 1939a and 1939b) seem to indicate clearly that the main channels of transport for organic materials, and also of certain viruses are the sieve-tube elements. As to the mechanism of this movement in sieve-tubes there is, however, considerable disagreement. Crafts (1939a) on the basis of a wide series of recent studies on this problem has developed a "pressure flow" theory which he summarizes as follows: "In the pressure flow mechanism, solvent and solute are assumed to flow together as a solution through elements of specialized structure or permeability, the protoplasm of which plays an entirely passive role in the process. The sources of energy in this mechanism lie in the osmotic activity of the products of assimilation in green portions of the plant and in the accumulative ability of cells in growing and storing tissues." Other explanations of the mechanism of transport in sieve-tube elements assume that protoplasmic streaming or highly-active protoplasm is concerned in some way with the process. One result of the lively interest in the function of phloem has been a series of exploratory studies on the structure and development of sieve-tube elements (cf. Esau, 1938). The recent literature in this field, as well as the historical background of the various problems, have been discussed recently by Esau

(1939) and the following resumé on sieve-tube elements is intended simply as an introductory guide.

1. *The protoplast of sieve-tube elements.* One of the most typical characters of the *mature* sieve-tube element is the *absence of a nucleus.* Numerous developmental studies (cf. Esau, 1939, p. 375 and 1941, pp. 452-454, Pl. 7) have shown that while the *young element* possesses a normal nucleated protoplast, maturation is accompanied by the eventual disintegration of the nucleus. Prior to its breakdown, the nucleus has been observed to increase significantly in size and to lose its chromaticity. Crafts (1939a, p. 176), in particular, has stressed the physiological significance of the enucleate condition in the sieve-tube element as follows: "The whole history of the sieve-tube portrays the intimate relation of the nucleus to the structure and function of the elements. No student of ontogeny can fail to sense the influence that the loss of nucleus has upon subsequent activity. From the beginning of its functioning period to its death, the sieve-tube element is doomed to a passive rôle, conditioned by its lack of nucleus and consequent permeability." A peculiarity of the cytoplasm of mature sieve-tube elements consists, according to the investigations of Crafts, in its highly permeable nature. Following the disappearance of the nucleus, Crafts finds that cytoplasmic streaming ceases and the cytoplasm "fails to plasmolyse in hypertonic solutions." Furthermore, the cytoplasm in the mature element loses its former ability to accumulate neutral red, a vital stain. Crafts (1939a, p. 175) interprets these facts as indicating the highly permeable character of adult sieve-tube elements. As Esau (1939, p. 403) has pointed out, the enucleate and permeable cytoplasm of mature sieve-tube elements is not to be regarded as "dead." This appears to be demonstrated by the continued deposition of callus on the sieve-plates during late stages in ontogeny. The contents of mature sieve-tube elements of certain species (e.g., *Cucurbita*) consist of slimy proteinaceous material which, in sections of phloem treated with heat or alcohol, may coagulate on the sieve-plates and in the lumen to form funnel-shaped structures known as *slime-plugs.* (Cf. Eames and MacDaniels, pp. 193-194, Figs. 90C and 91C; and Esau, 1939, pp. 379-383). The most recent evidence supports the belief that

slime-plugs are *artifacts* rather than structures peculiar to normal uninjured sieve-tube elements. The slime in sieve-tube elements originates from the disintegration of the *slime-drops* which are commonly present in angiosperms as inclusions in the cytoplasm of young cells; the disintegrated nucleus and the sieve-tube sap also become part of the slimy contents of the sieve tubes. In many plants, *leucoplasts* and *starch grains* may be observed in the sieve-tube elements (cf. Esau, 1939, pp. 384-386).

2. *Sieve-plates and sieve-fields.* In addition to the ultimate loss of the nucleus, the mature sieve-tube element is characterized by the presence of *sieve-plates* which occur in various regions of the cell wall. The origin of the term "sieve-tube" rests upon the erroneous idea that, in such a plant as *Cucurbita*, the end-walls of the elements are perforated like a sieve. But modern studies agree in showing that sieve-plates are not literally open, perforated areas in the wall. On the contrary, the so-called pores in the sieve-plate appear to be penetrated by either delicate or rather coarse *plasmodesmata*. Crafts (1939a) has concluded that these cytoplasmic strands, which thus connect the protoplasts of adjacent sieve-tube elements, are solid rather than tubular in structure as was maintained by certain earlier workers. Considerable variation occurs with reference to the *distribution of sieve-plates* in sieve-tube elements. In highly specialized sieve-tubes (e.g., in *Cucurbita*), the transverse end-wall is occupied by a single large plate with rather coarse plasmodesmata while the lateral walls are provided with smaller, less distinct plate-like areas. But several sieve areas forming a "compound sieve-plate" may be present in end-walls which are inclined or sloping. In the gymnosperms, very numerous small sieve-plates or "sieve-pits" occur on the radial lateral walls (Abbe and Crafts, 1939). The term "*sieve-field*" was originally applied by Nägeli to the apparently reduced sieve-plates present on the lateral walls of angiospermous sieve-tube elements. However, as Esau (1939, p. 395) has clearly indicated, sieve-plates and sieve-fields may intergrade in structure so that only an arbitrary distinction can be made between them. The *morphological nature* of the sieve-plate is still, to some extent, an unsolved problem. This is largely the case because of the many gaps in our knowledge as to the

method of development of these structures. Furthermore, the terminology used in describing the adult sieve-plate and its homologues is, as Esau (1939) has shown, in a confused state. It does seem evident, however, that in some respects, sieve-plates are fundamentally similar to simple pits (Esau, 1939, pp. 397-399). In conifers, the sieve-plates arise directly from the large primordial pits which are present on the radial walls of the young sieve-cells. Also in many angiosperms, it is possible to trace the origin of the sieve-plate to a primordial pit of a meristematic cell. In some plants, however, such as *Robinia* and *Cucurbita*, it does not appear possible to refer the large solitary sieve-plates on the end-walls to development from a single primary pit area. Esau suggests that in *Robinia* "one might assume that several shallow primordial pits together form one sieve-plate, the single or numerous plasmodesmata of one pit giving rise to one connecting strand of a sieve-plate." During the development of the sieve-plate, each plasmodesma or in gymnosperms each group of plasmodesmata becomes surrounded by a cylinder of *callus*. The chemical nature of this substance is still in question but callus-cylinders are readily stained and differentiated by treating sections with *aniline blue*. As the maturation of the sieve-tube element progresses, the amount of callus on the sieve-plates increases so that the originally separate cylinders become confluent or fused and the plate becomes coated on both sides with callus. According to Esau (1939, p. 391) this final accumulation is "*definitive-callus*" and indicates "the approach of a functionless state of the sieve-tube." There appears to exist no conclusive evidence that definitive-callus blocks up the pores of the sieve-plate. On the contrary, the plasmodesmata are noticeably stretched and eventually die. In many plants, the definitive-callus becomes dissolved away from the plate prior to the death of the sieve-tube elements and their companion cells which eventually become obliterated or crushed by neighboring cells.

3. *Lateral walls of sieve-tube elements.* The lateral walls of recently differentiated sieve-tube elements are frequently thick and glistening in untreated sections. They have been termed "*nacre*" because of this appearance. Chemically, these walls appear to consist of cellulose and probably are, morphologically,

primary walls. In various members of the Abietineae, however, Abbe and Crafts (1939) reported true secondary walls in the sieve-elements of the secondary phloem. According to Esau's review, the nature of the pits or protoplasmic connections of sieve-tube elements with companion cells and phloem parenchyma cells is not yet definitely established. In some cases at least, the wall between the sieve-tube element and its companion cell is penetrated by numerous scattered plasmodesmata.

II. Material for the Study of Sieve-Tube Elements.—

1. *The phloem of Cucurbita.* Obtain a thin transverse section of the stem of pumpkin and examine its structure under low magnification. In progressing from the edge of the section towards the center the following tissues may be observed, viz.: (1) a typical uniseriate *epidermis*, certain cells of which have developed into hairs; (2) a rather narrow *cortex*, consisting of an outer, discontinuous zone of *angular collenchyma* followed by a region of *parenchyma* the innermost layer of which may contain abundant starch grains and appear as a *starch-sheath* if the section is stained in iodine; (3) the *stele*, the outer boundary of which is clearly indicated by a continuous cylinder of thick-walled *pericyclic fibers*. Internal to the fibers occurs a broad parenchymatous zone in the inner portion of which are found two "rings" of *vascular bundles*. Each bundle consists of a median strand of *xylem* (characterized by its large vessels) flanked on both sides by a strand of *phloem*. A bundle of this type is designated as a *bicollateral vascular bundle*. Clear evidence of a *cambial zone* may be seen between the xylem and each of the phloem strands, especially in the larger inner bundles. The center of the stem is occupied by an irregular cavity which was produced by the collapse and disintegration of the pith. *To investigate the structure of the phloem*, remove the cover-glass and mount the section in a .1% aqueous solution of aniline blue.[1] This dye will stain the callus depositions on any of the sieve-plates which may be present in the section. A careful study, *under high magnification*, of the phloem of the various bi-collateral bundles will usually reveal a number of large *sieve-plates*. These structures in *Cucurbita* occupy virtually the entire end-

[1] Cf. Appendix, p. 142.

wall of the cylindrical sieve-tube elements. In critically stained sieve-plates, each "pore" is occupied by a dark central spot, representing the large *plasmodesma* and is surrounded by a distinct *callus-cylinder* stained a light blue. If *definitive-callus* has not yet appeared, the portions of the plate between the callus-cylinders should appear unstained. Note that in addition to the sieve-tubes, smaller *companion cells* appear and are distinguished by their nucleated protoplasts and their triangular or quadrangular form as seen in trans-section. *Phloem parenchyma* is also present but is difficult to distinguish from sieve-tube elements unless the latter exhibit sieve-plates. This study of phloem should be continued with the aid of *longi-sections* which likewise should be stained in aniline blue. Note carefully the appearance and structure of the sieve-plates as seen in sectional view, and the relation of the sieve-tube elements to the companion cells and the phloem parenchyma. Often *slime-plugs* will appear in certain of the sieve-tube elements. They can readily be induced by treating the section with 70% alcohol.

2. *The phloem of Robinia and Pinus.* Mount thin radial and tangential sections cut from living twigs of these genera in a .1% aqueous solution of aniline blue and study under high magnification the structure and distribution of the sieve-plates and the form and relationship of the sieve-tube elements and sieve-cells.

III. Suggested Drawings and Notes.—

1. Diagram the general structure of a bicollateral bundle from the stem of *Cucurbita* as seen in trans-section. Label all essential parts and indicate by circles the position of the largest vessels of the xylem.

2. Draw small portions of the phloem tissue of *Cucurbita* as seen in both trans- and longi-sectional views. Show carefully the structure of at least one sieve-plate in each drawing. Label all cell types and structures.

3. Prepare drawings of small portions of the phloem of *Robinia* and *Pinus* to show the form and structure of the sieve-tube elements and sieve-cells.

4. Summarize the evidence which indicates that plant viruses may move and be transmitted through phloem tissue (cf. Crafts, 1939b, and Esau, 1941).

5. Outline briefly the experimental evidence as to the role of the phloem in the trans-location of organic solutes (cf. Crafts, 1939b).

REFERENCES

1. Abbe, L. B., and Crafts, A. S., Phloem of White Pine and Other Coniferous Species. Bot. Gaz. 100:695-722. 1939.
2. Crafts, A. S., The Relation between Structure and Function of the Phloem. Amer. Jour. Bot. 26:172-177. 1939a.
3. ————, ——, Movement of Viruses, Auxins, and Chemical Indicators in Plants. Bot. Rev. 5:471-504. 1939b.
4. Eames and MacDaniels, pp. 69-76 and Ch. VIII.
5. Esau, K., Ontogeny and Structure of the Phloem of Tobacco. Hilgardia 11:343-424. 1938.
6. ————, ——, Development and Structure of the Phloem Tissue. Bot. Rev. 5:373-432. 1939.
7. ————, ——, Phloem Anatomy of Tobacco Affected with Curly Top and Mosaic. Hilgardia 13:437-490. 1941.
8. Hayward, pp. 614-616.

Exercise XII

THE STEM

I. Introduction.—In this and the two following exercises, a brief study will be made of the comparative anatomy of the three principal vegetative "organs" of the sporophyte of seed plants, viz.: the stem, leaf, and root. Paleobotanical evidence shows clearly, however, that this conventional subdivision of the plant body cannot be applied to the Psilophytales which are generally regarded as the most primitive of all tracheophytes. Indeed, in the Psilotales, which are the living representatives of this ancient group, roots are absent and the aerial portion of the sporophyte is not clearly demarcated into stem and leaves. Furthermore, it is clear that even in seed plants the boundary between stem and leaf can only be made rather arbitrarily. Both of these "organs" arise from a common terminal meristem (i.e., the shoot apex) and their further differentiation and growth is reciprocal and interdependent to a large extent. For these reasons, it seems preferable, from a morphological standpoint, to include both the axis (i.e., the stem) and its foliar appendages under the broader concept of the *shoot*. This concept has found application not only to the vegetative region but has also been widely adopted in the anatomical interpretation of the flower of angiosperms (cf. Eames, 1931, and Foster, 1939). Hence, in this book, the separate treatment given to the stem and the leaf is largely a matter of practical convenience and its limitations on morphological grounds should be constantly borne in mind. (Cf. Arber, 1941, for a penetrating discussion of the problem.) The *root*, which is axis-like in form, clearly deserves separate discussion and study and its anatomical features will be outlined briefly in Exercise XIV.

In the following resumé of the basic aspects of stem anatomy, the histology of the *internodal regions* of this structure is the principal consideration. The *nodal regions* of stems, because of

the complications in vascular anatomy resulting from the development of leaves and buds, offer a series of problems which are beyond the scope of the present book. In general, however, it may be stated that in gymnosperms and dicotyledons, the cylinder of vascular tissue is interrupted at or near the node by the development (*early in ontogeny*) of *leaf gaps*, which are parenchymatous areas in the siphonostele situated above the point of divergence of the *leaf traces*. Depending upon the nature of the foliar structure as well as upon the plant, the anatomy of the node is described as *unilacunar* (one gap), *trilacunar* (three gaps) or *multilacunar* (more than three gaps). There appears to be some evidence that in angiosperms the trilacunar node is the primitive condition. (For further details cf. Eames and MacDaniels, pp. 114-120.) The development of the axillary bud results in additional complications in the vascular anatomy of the node. The earliest vascular bundles of the bud are known as *branch traces* and their "divergence" from the main axis likewise is associated with parenchymatous areas in the stele which are termed *branch gaps*. Wide application of the principals of nodal anatomy has been made in the study of the vascular anatomy of the flower (cf. Eames, 1931, and Wilson and Just, 1939).

1. *The primary structure of the stem.* In many of the lower vascular plants and in certain herbaceous angiosperms (particularly monocotyledons), all the stem tissues are primary, i.e., they originate directly from the *progressive differentiation* of cells derived from the apical meristem of the shoot. Despite the great variation in the kinds and patterns of tissues, a common plan of primary structural organization is found in the stems of most gymnosperms and angiosperms. This consists in the existence of three more or less well-demarcated zones or regions which now may be described briefly as follows:

(*a*) *The epidermis.* Stems possess a well-defined epidermis in which stomata and various types of trichomes may be present in addition to typical epidermal cells (cf. Exercise V).

(*b*) *The cortex.* Beneath the epidermis of stems is found a cylindrical zone, variable in its radial dimension and in the kinds of cells which occur. This region is the cortex and in the simplest

condition is formed of thin-walled *parenchyma tissue*. Often, however, the cortex is more complex histologically and exhibits an outer, subepidermal area of *collenchyma* (which occurs as a continuous cylinder or as separate strands) and an inner region of parenchyma. Other cell types may also appear in the cortex, particularly *sclereides*, *fibers* and *secretory cells*.

(*c*) *The stele*. This region of the stem includes the *primary vascular tissues* as well as variable amounts of parenchyma.

Since the original formulation of the "Stelar Theory" by Van Tieghem and Douliot in 1886, comparative studies on the stem have largely centered upon the organization and phylogeny of the stele, particularly with reference to the distribution of phloem and xylem and the morphological nature of the pith. It is now rather generally held that two principal *types of steles* occur in the sporophyte of vascular plants, viz.: (1) the *protostele*, which consists of a central core of xylem ensheathed by phloem, and (2) the *siphonostele* which is characterized by the presence, internal to the protoxylem, of a central mass of parenchyma known as the *pith*. The evidence from comparative anatomy, including the facts of paleobotany, supports the idea that the protostele is the primitive type. This type of stele is found in *both* the stem as well as the root of many lower tracheophytes but is restricted to the root of seed plants. The *order of maturation* of the tracheary elements of the xylem in a protostele is *centripetal* and primary xylem of this kind is termed *exarch*. In contrast, the protoxylem in a siphonostele is situated at the outer edge of the pith and the order of maturation of the xylem is *centrifugal*. *Primary xylem* in the stems of seed plants is thus *endarch*. A discussion of the involved controversy as to the way in which the siphonostele may have originated from the protostele is beyond the scope of this book and the student is referred to the excellent resumés given by Eames and MacDaniels (pp. 112-114 and 337-340) and Smith (1938, pp. 124-131). In the writer's opinion, however, the evidence from ontogeny appears to favor the idea that at least in seed plants the pith region is morphologically a part of the stele and represents the parenchymatization of potential vascular tissue. As seen in transsection, the vascular tissue of the siphonostele appears either as

a continuous cylinder, broken only by the short leaf gaps in nodal regions, or as a "ring" of vascular bundles. A stele of the latter type should be visualized as a *tubular network*, the "meshes" representing the long vertical leaf gaps or parenchymatous rays. This kind of stele is termed a *dictyostele* and its component bundles are either *collateral* or *bicollateral*. In gymnosperms and dicotyledons, the siphonostele is specifically designated as *ectophloic* if there is only an external area of phloem, or as *amphiphloic* if both internal as well as external phloem occurs. This latter type of siphonostele is restricted to certain families in the dicotyledons and also appears in the stems of some ferns. The nature of the *boundary* between stele and cortex has produced much discussion among anatomists. A common view is that the *endodermis* represents the innermost layer of the cortex and that hence all tissues internal to it, including the *pericycle*, *vascular tissues* and *pith*, constitute the stele. Such a demarcation is practical in roots which typically develop a well-defined endodermis, and the evidence of ontogeny indicates that at least in some plants, the endodermis is morphologically a part of the cortex (cf. Esau, 1941). In the stems of seed plants an endodermis or its equivalent (i.e., the so-called "*starch sheath*") may be present. Under such circumstances it should be clear that until further ontogenetic evidence becomes available, only an approximate and somewhat arbitrary demarcation can be made in many stems between the innermost region of the cortex and the adjacent pericycle.

2. *The ontogeny of the stele in vascular plants.* Within recent years there has been a marked revival of interest in the origin and differentiation of the primary vascular system. Among the more important contributions may be cited the work of Helm (1932), Barthelmess (1935), Louis (1935), Kaplan (1937), and Grégoire (1938). One of the principal objectives in these investigations has been to determine how the provascular tissue or procambium is produced from the tissue of the shoot apex. In view of the involved aspects of this process, it will only be possible to outline certain important steps.

In a number of dicotyledons, the first stage in the determination of the *position* of the provascular tissue consists in the early

differentiation of the *pith*. This sets apart, near the base of the shoot apex, a *peripheral zone* of tissue which shows little or no evidence of specific differentiation. The next step, as one proceeds away from the summit of the apex, is concerned with the effects produced by the developing leaf primordia. These structures early assume a dorsiventral character and their abaxial regions begin early to develop as parenchymatous tissue. As a result, at least in many dicotyledons, trans-sections below the base of the shoot apex, show a more or less distinct "ring" of embryonic tissue demarcated on the outside by the united bases of the leaves and on the inside by the developing pith. This tissue zone has been designated by Helm as the "meristem ring" and by Louis as the "prodesmogen." From it there is produced the provascular or procambial cells which ultimately give rise to the primary vascular system of the stem.

Divergent ideas are held as to the *way* in which procambium arises from the meristem ring or prodesmogen. According to one view, the procambium arises as isolated strands in the bases of the young leaf primordia, from which points its further differentiation proceeds acropetally towards each leaf apex and basipetally towards the prodesmogen tissue in the axis. However, a number of recent studies (e.g., Boke, 1940, 1941) suggest that this may be an erroneous interpretation and that the development of provascular tissue in the region of the shoot apex may be exclusively acropetal.

One of the important aspects of recent studies has been to emphasize the interrelationship which exists between stem and leaf in the building up of the stele. This is shown by the fact that in conifers and many dicotyledons, the first vascular bundles in the young siphonostele are *leaf traces* or so-called "common bundles." The *interfascicular areas* in the "meristem ring" may either produce additional procambial tissue from which additional primary xylem and phloem arise, or as in dictyosteles, progress towards the formation of parenchyma. In the latter case, the interfascicular strips either mature as typical *medullary rays* or give rise to an *interfascicular cambium*.

The question of the differentiation of primary phloem and primary xylem in leaf traces has apparently received only meagre

study. According to the work of Esau (1938, p. 396) on tobacco, the primary xylem differentiates both upwardly into the leaf and downwardly into the axis. But "the phloem of a leaf trace follows a different course of development from that of xylem. It differentiates, at least in the species considered in these studies, acropetally from the stem into the leaf."

3. *The secondary structure of the stem.* In gymnosperms and many angiosperms, the so-called primary tissue regions are relatively short-lived and eventually are destroyed or embedded by the development of *secondary tissues.* As pointed out in Exercise X, the distinction between primary and secondary vascular tissues is rather arbitrary and depends fundamentally upon the way in which "procambium" is distinguished from the "vascular cambium." The process of secondary growth in stems is extremely complex and the following outline is intended merely as an introductory guide. (For a detailed treatment cf. Eames and MacDaniels, Ch. VI.)

The *vascular cambium*, theoretically regarded, is a uniseriate meristem composed of *ray initials*, which produce the *vascular rays*, and *fusiform initials* which give rise to the "vertical" cell types in the secondary phloem and secondary xylem. In examining developing siphonosteles *in trans-section*, however, it proves difficult or impossible to distinguish the cambial initials from their most recent derivatives. For this reason, the term "cambial zone" may be used collectively to designate the cambial initials and their adjacent undifferentiated phloem and xylem mother cells. The vascular cambium may be thought of as the direct continuation of the undifferentiated procambial tissue situated between the metaxylem and the metaphloem. If one is concerned with a dictyostele, a distinction is made between the cambium within each bundle (the *fascicular cambium*) and the cambium which arises from parenchyma—like tissue between the bundles (*interfascicular cambium*). The latter may produce additional phloem and xylem or as in certain vines, broad rays of "secondary" parenchyma. The effects of sustained cambial activity upon the primary tissue regions is profound. All extra-cambial tissues, i.e., primary phloem, pericycle, cortex and epidermis, are affected and eventually, through the added activity of the

phellogen or cork cambium, are sloughed away. Their place is gradually taken by the *bark* which consists in many gymnosperms and dicotyledons of dead or dying secondary phloem and of areas of *periderm*. The intra-cambial primary tissues, i.e., primary xylem and pith, are completely buried within the cylinder of secondary xylem and, as a result of stretching and compression, may be crushed or even destroyed.

The early phases in development of secondary vascular tissues in woody stems are usually accompanied by the formation of a *periderm* or corky tissue beneath the epidermis. Functionally, the periderm acts as a protective layer, replacing in this respect the epidermis which is eventually killed and sloughed away. Structurally, the term periderm is applied to the *phellogen* or cork cambium and its two derivative tissues, viz.: cork or *phellem*, and *phelloderm*. The first-formed phellogen in the stem appears to arise as a result of the regressive differentiation of epidermal, cortical or pericyclic parenchyma cells. Its initiation is indicated by the tangential division of certain cells. In some species, these first tangential divisions appear in the epidermis. Most commonly, perhaps, the phellogen originates in the outermost cells of the cortex. There is evidence that in some stems, the cortical phellogen first makes its appearance beneath the stomata, at which point *lenticels* are produced. Continued spread of the phellogen from these structures may result in the formation of a cylinder of cork cambium. As a result of the repeated tangential division of the phellogen cells, the derivative tissues exhibit alignment of the cells in radial rows. Cells differentiating towards the outside of the phellogen lose their protoplasts, acquire *suberin* in their unpitted walls, and finally mature as cork cells. The phelloderm tissue, which is usually much less in extent than the cork, originates from the inner derivatives of the phellogen. *Phelloderm cells* are described as being parenchyma-like in retaining their protoplasts and in having simple pits in their cellulose walls. The functional life of the first-formed phellogen in woody stems is short and new layers of cork cambium arise successively from deeper regions of the cortex and pericycle until, finally, the living cells of the secondary phloem participate in periderm formation. The ultimate result is the production

in many species of shell-shaped layers of periderm which enclose masses of dead or dying phloem tissue (cf. Eames and MacDaniels, p. 212, Fig. 96).

The first-formed as well as later developed periderm layers in stems are usually provided with *aerating structures* termed *lenticels*. As stated above, lenticels are usually initiated by the appearance of a phellogen beneath a stoma (cf. Eames and MacDaniels, p. 219, Fig. 100). In the development of a lenticel, the phellogen, instead of producing typical cork, forms a mass of loosely-arranged cells with unsuberized walls which make up the *complementary tissue*. This tissue in many lenticels may be subdivided by layers of smaller more compact cells which are termed *closing layers*. The pressure exerted by the outwardly-developed mass of complementary tissue is sufficient to rupture the epidermis which, together with the underlying layers of adjacent cork, curls back from the edges of the lenticel as flaps of broken tissue. In many plants (e.g., *Sambucus*), the extruded complementary tissue is very prominent. According to De Bary (p. 561) the puffy swelling of lenticels in trees during wet weather may be the result of the "hygroscopicity" of the complementary tissue.

II. Material for the Comparative Study of the Stem.—The choice of material for the study of stem anatomy will depend upon the forms available as well as upon the points to be illustrated. The following stem types have proved useful and are recommended. Free-hand sections of stems stained with phloroglucinol and hydrochloric acid are of considerable use. But for the finer details of structure and development, permanent mounts of critically stained sections are necessary.[1]

1. *The stem of the geranium* (*Pelargonium* sp.). Examine a *transverse section* of the stem, and study the following tissues and regions from the edge of the section inwardly, viz.:

(*a*) The *epidermis*, a uniseriate layer of small oval or elliptical cells with thick inner and outer walls (the latter covered with a thin *cuticle*) and somewhat thinner radial walls. A protoplast, which may appear somewhat collapsed, should be evident in most cells. Certain of the epidermal cells have given rise to *stomata*

[1] Cf. Appendix, p. 140.

while others have developed to various types of trichomes, including glandular capitate hairs and multicellular unbranched hairs, the latter similar to the hairs already studied on the geranium leaf. (Refer to Exercise V.)

(*b*) Immediately within the epidermis is found the *cortex*, a region composed of twelve or more layers of rather typical "isodiametric" parenchyma cells which are separated from one another by prominent intercellular air spaces. A protoplast is present in many of these cortical cells, the main functions of which are photosynthesis and food storage, as is evidenced by the frequency of starch grains in these cells; druses of calcium oxylate are found in many of the cortical cells, occupying nearly the whole cavity of the cell.

Depending largely on the distance from the shoot apex at which the sections on your slide were taken, you will find the early or later stages in the development of *cork* or *phellem*. In studying the phellem in the stem of the geranium, notice that *druses* are occasionally found in some of the inner cork cells. If your sections show a phellem four or five layers in thickness, you will find that the tangential (and to some extent the radial walls) are somewhat wavy or irregular. This condition, which is very commonly found in corky tissue, results from the constant pressure of the successively developing and enlarging cork cells upon the older cells of the phellem. *Typical phelloderm, however, is rarely developed in the young stem of the geranium.*

(*c*) Directly inside the innermost layer of cortical cells occurs the *stele*, the outer region of which is indicated by the pericycle which in the geranium stem consists of an outer continuous ring of thick-walled *pericyclic fibers* (elongate tightly joined cells, as seen in longitudinal section, with characteristic slit-like spirally-arranged pits) followed by several layers of small thin-walled *parenchyma cells*. The *vascular tissue* of the stele lies directly within the pericycle and consists of a ring of *typical collateral bundles* (i.e., bundles in which the phloem lies *radially external* to the xylem) which are separated from one another, at least in certain regions, by strips or bands of parenchymatous tissue which because of their *direct* connection with the pith (medulla) may be termed *medullary rays*.

In view of the obvious variation in the size and degree of development of the vascular bundles in any given section, the following description is only general in nature; and all essential variations and details must be indivdually interpreted.

A well-developed *vascular bundle* in the geranium stem is somewhat wedge-shaped or triangular in cross-section and consists of an external region of phloem tissue separated from the internal region of xylem tissue by the *cambial zone* in which cell divisions occur predominately in the *tangential plane*.

Beginning with the phloem tissue of the bundle first of all, you should be able to find the *somewhat crushed primary phloem* lying directly against the innermost cells of the pericycle; structurally, primary phloem in the geranium stem appears to consist of small sieve-tubes, companion cells and phloem parenchyma. Lying directly inside of the primary phloem occurs the *secondary phloem* which is composed of (1) *sieve-tubes*, rather large polygonal cells, apparently devoid of contents; (2) *companion cells*, extremely small, more or less *triangular cells* (in cross-section of course) *closely joined* to the sieve-tubes and usually containing a definite nucleated protoplast; and (3) large thin-walled *parenchyma cells*.

Separated from the secondary phloem by the "cambial zone" occurs the xylem tissue of the vascular bundle. In all of the larger bundles, the xylem is of two kinds, viz.: (1) *secondary xylem*, an external layer of thick-walled isodiametric, tightly joined cells which are arranged in more or less definite radial rows because of their origin from the *fascicular cambium;* and (2) the *primary xylem*, an internal group of rather large, more or less polygonal cells, irregularly arranged and imbedded among isodiametric thin-walled parenchyma cells. The smallest cells of the primary xylem (which has differentiated centrifugally from the procambial strand) are found nearest the *inner edge* of the vascular bundle and represent the first formed elements of the *protoxylem*. Notice particularly that in many protoxylem cells, portions of the spiral thickening have been torn from the wall in the process of sectioning; in other cells, a piece of the spiral band may be seen projecting into the lumen of the cell.

From what has already been said of the structural characteristics of the tracheary cells of the xylem, it is almost unnecessary to state that the limits between secondary xylem and metaxylem are virtually impossible to determine when considering only transverse sections of a vascular bundle. In the interval between each of the individual bundles in the ring, there occurs a zone of small thin-walled, obviously meristematic cells which are *continuous* with the cambial zone in the bundles themselves and represent what is termed the *interfascicular cambium*. The interfascicular cambium originates by the tangential division of cells in the medullary rays adjacent to the strips of fascicular cambium. In certain plants, the interfascicular cambium only forms parenchyma (e.g., *Clematic, Aristolochia*) but in *Pelargonium*, as in many herbaceous plants, the interfascicular cambium gives rise to additional "bundles" composed entirely of secondary phloem and secondary xylem; the extensive development of vascular tissue from the interfascicular cambium usually results in the formation of a complete cylinder of secondary xylem and phloem. In studying the sections note that phloem is the first vascular tissue to differentiate from the interfascicular cambium and is later followed by the centripetal formation of secondary xylem; whether this is an exceptional condition can only be determined by the investigation of a large number of herbaceous stems.

The center of the stele is occupied by the *pith*, which is composed exclusively of large isodiametric thin-walled *parenchyma cells* separated from one another by conspicuous intercellular airspaces. The extreme abundance of *starch grains* in the cells of the pith indicates that this region has as its function the storing of reserve food material.

2. *The stem of the basswood or linden* (*Tilia* sp.). Obtain a stained slide with transverse, radial and tangential sections of the stem. Examine first the trans-section and study the following tissues and regions from the periphery of the stem to its center, viz.:

(*a*) The *epidermis*, a uniseriate layer of cells which appears broken and cracked in numerous places due to the development of a prominent *periderm* beneath it. Structurally the cells of the epidermis are rather small, are oval in shape (in section view)

and possess thick, slightly stratified outer walls overlaid by a *cuticle;* in most instances, inclusions and the remains of the disintegrated protoplast are present in the epidermal cells.

(*b*) Immediately within the epidermis occurs the *periderm* which is composed of three layers of tissue, viz.: (1) the *phellem* or cork which is differentiated centrifugally from the phellogen and consists of a varying number of layers of narrow, laterally compressed cells which are arranged in definite radial rows and are densely packed (except the two outermost layers) with dark brown material which consists probably of substances classed under the general head of *tannins;* (2) the *phellogen* which is a uniseriate layer of meristematic cells found next to the innermost layer of phellem cells. In *Tilia*, as in so many woody stems, the phellogen is initiated by the tangential division of the outermost layer of cortical cells. (3) Internal to, and directly next to the phellogen occurs a single layer of cells characterized in this instance by their rectangular form and obvious protoplasts. This layer of cells is known as the *phelloderm* and differentiates centripetally from the phellogen. Note that the corky layer of the stem is definitely broken at certain points which appear as somewhat lens-shaped areas; these regions are known as *lenticels.* In studying the structure of the lenticels of the basswood stem, observe particularly the continuity between the phellogen and phelloderm of the lenticel and these tissues as they occur at either side of the lenticel. In this particular developmental stage of the stem in *Tilia* the lenticels have been formed from the first or primary cork cambium; as the stem increases in diameter from year to year, new lenticels are formed at various points on the surface of the bark by the activity of subsequently formed phellogen layers; thus even in relatively old branches, lenticels develop and represent the necessary areas through which exchange of gases between the living tissues and the atmosphere can take place in the essential process of respiration.

(*c*) Directly internal to the periderm occurs the *cortex*, which consists of two rather definite regions, viz.:

(*i*) An outer region composed of four or more layers of *collenchyma* cells, which in the position of their thickened primary walls appear intermediate in character between "angular"

and "lamellar" collenchyma. Notice that intercellular air spaces are extremely small and difficult to distinguish.

(*ii*) An *inner region* composed of typical "isodiametric" *parenchyma cells* with active protoplasts and cells containing druses of *calcium oxalate*. The latter type of cells tend to occur in groups (refer to the radial section of the stem) and their specialized character has earned for them the term of "*crystal sacs.*" It is important to notice the crushed appearance of many of the parenchyma cells in the cortex; this condition has been caused by the pressure of the secondary phloem which is constantly pushed toward the periphery of the stem.

(*d*) *The stele.* *Tilia* possesses an *ectophloic siphonostele* but the arrangement of the tissues of the secondary phloem shows a number of features distinctive of this genus. The phloem occurs just within the innermost layer of the cortex and consists of a cylinder made up of wedges of tissue showing a characteristic "banded" appearance which alternate with triangular sectors which are "homogeneous" in structure; the wedges showing the band-like structure are broadest next to the cambium while just the reverse relationship obtains in the case of the homogeneous sectors.

Examine in detail, first of all, the structure of one of the large "*banded*" *sectors.* You will observe that the characteristic "banded" appearance of such a sector is due to the alternation of tangential layers of extremely thick-walled fibers with layers of thinner walled cells. These thick-walled *phloem fibers*, which provide mechanical support and flexibility to the stem, have long been known as "bast fibers" and their prominent development in the genus *Tilia* is responsible for the old English name of this tree, i.e., "bast-wood" which in modern language has been changed to "basswood." Under high power, the phloem or "bast fibers" appear irregularly polyhedral, are closely joined without evident intercellular air spaces, and possess extremely small lumina; under especially favorable conditions of magnification and illumination you may be able to see the infrequent canal-like pits between adjacent cells.

The layers of thinner-walled cells alternating with the bands of "bast fibers" are composed of the conducting and storage cells

of the phloem, viz.: radial rows (one or occasionally two cells in thickness) of living parenchyma cells formed from the cambium and extending out to the cortex through the phloem; these radial rows of cells (which of course are actually sheets of tissue) are the *phloem rays* and are directly continuous across the cambium with corresponding rays of the secondary xylem. The phloem rays function both in radial transportation as well as in the storage of food materials. Starch grains are often present in these cells. The *sieve-tubes*, which are the important vertical conducting elements of the phloem, appear in transverse section as large somewhat irregular thin-walled cells which appear devoid of protoplasts and are closely joined on their smaller wall-facets with the *companion cells*. The latter are very small, appear more or less triangular in transverse section and in most cases possess definite nucleated protoplasts. Interspersed among the sieve-tubes are the small isodiametric *phloem parenchyma cells* which possess a living protoplast.

Turning now to the wedges of "*homogeneous tissue*," it will be found that these sectors are entirely composed of somewhat "rectangular" parenchyma cells, most of which are provided with a definite protoplast. The characteristic form of these sectors is due to a *process of dilatation* of the multiseriate phloem rays lying between the "banded" sectors of the stem. In other words, certain of the phloem rays (usually those which are two or three cells in width at the cambium) increase continuously in width by the radial and tangential division of the parenchyma cells; the intervening areas of phloem (i.e., those containing the bast fibers and the sieve-tubes) are thus separated from each other and correspond in position to the phloem portions of the original vascular bundles. As the stem increases in age, a similar process of dilatation occurs in the smaller rays of the phloem with the result that the "banded" sectors become successively subdivided into smaller groups (for complete details, cf. De Bary pp. 536-537). It should be noted that these *dilated phloem rays* are directly continuous across the cambium with bi- or triseriate xylem rays. *Druses* occasionally appear in the parenchyma cells of the dilated phloem rays.

The "*cambial zone*" occurs directly between the secondary xylem and the secondary phloem and consists of the *uniseriate cambium* itself and a varying number of layers of "maturing" vascular elements. A careful study of the "cambial zone" will help in understanding the direction of formation of secondary xylem and secondary phloem and will furthermore shed some light on the nature of the early developmental stages of the cells making up these tissues.

Within the "cambial zone" occurs the cylinder of *secondary xylem* which is composed of two or three more or less concentric layers or cylinders of xylem tissue, each of which is known as an *annual ring*. In all woody stems of north temperate plants, an annual ring represents the amount of secondary xylem formed during a single growing season. The presence of annual rings in the secondary xylem of perennial woody plants seems to be determined to some extent by seasonal conditions, since the xylem tissue formed in the spring (the so-called "*spring wood*") differs somewhat in respect to the size, type and arrangement of its cells from that formed in the summer (the so-called "*summer wood*"). It is due to this structural difference between "spring" and "summer" wood that an annual ring appears distinct. In many cases, the cells formed during late summer tend to be somewhat smaller and thicker-walled than those arising in the spring; often the difference is emphasized by the localization of the majority of the vessels in the spring wood.

The secondary xylem of *Tilia* is, like that of many woody dicotyledons, a very complex "tissue system," consisting of the following types of cells:

(*a*) *Vessel elements*, which are large prominent cells, rather polygonal in transverse section, and possessing large empty lumina. In especially thin regions of your section you may be able to see small bordered pits in cross-sectional view. Careful focusing should reveal the "compound middle lamella."

(*b*) *Tracheids*, which are smaller in size than the vessels and are often more or less rectangular in shape as seen in transverse section. In contrast to the vessels, the tracheids are frequently arranged in definite radial rows.

(*c*) *Fibers*, which are somewhat irregular in shape and are usually much smaller than either the vessels or tracheids and are usually provided with thicker walls.

(*d*) Scattered among the vessels, tracheids and fibers, occur the *wood parenchyma cells* which are small in size, isodiametric in form, and possess a definite protoplast. It should be realized that wood parenchyma cells occur in "vertical chains" or rows and function primarily in the storage of certain carbohydrates, particularly starch; the function of wood parenchyma in "assisting" the trans-location of substances in the xylem is imperfectly understood. In *Tilia*, the wood-parenchyma has no definite distribution in the xylem and is hence termed "diffuse."

Extending radially through the secondary xylem are the *rays* which are of two types, viz.:

(*a*) Large rays, which represent the xylem "extension" of the large specialized dilated rays of the phloem discussed previously. In the second annual ring, these large rays may be two or three cells in width but, at least in the inner portion of the first annual ring, they become a single cell in width and finally terminate directly in the pith. Such rays have been termed "primary medullary rays."

(*b*) *Small rays*, one cell in width which are the xylem extension of the rays extending through the "banded sectors" of the phloem. These rays have been termed "secondary medullary rays" but in some instances at least have no direct connection with the pith.

The *primary xylem* occurs next to the pith and is completely surrounded externally by the cylinder of secondary xylem. It is naturally very difficult to distinguish between the *metaxylem* and the first-formed elements of the secondary xylem in a transverse section of the stem of *Tilia*. The *protoxylem*, however, is quite distinct and appears as solitary or grouped thick-walled cells which are imbedded among the small parenchyma cells of the primary xylem at the periphery of the pith. The *pith* is a solid rod of tissue occupying the center of the stem. In contrast to the condition in the geranium stem, the pith of *Tilia* is not homogeneous but shows a certain amount of tissue specialization as follows:

The *periphery of the pith* is formed of several layers of rather thick-walled more or less isodiametric parenchyma cells which are provided with protoplasts and in addition contain very small starch grains; other of these peripheral cells are filled with dark-staining granular material, the exact nature of which is obscure at present. The bulk of the pith is composed of large "isodiametric" parenchyma cells which are separated by definite intercellular air-spaces; some of these cells likewise contain small starch grains and protoplasts. Interspersed among these large pith cells occur much smaller thicker-walled parenchyma cells which are filled with granular dark-staining bodies; these latter cells are found singly or in groups, but in longi-section appear in scattered vertical series.

Attention must finally be directed to the *mucilage-containing cells* which are arranged in a more or less definite ring near the periphery of the pith. These *mucilage-containing cells* may be identified by their large size, by the disorganized reddish or purple material found in them, and by the jacket of starch-containing parenchyma cells which surrounds each of them. The function of the mucilage formed in these cells is unknown.

3. *The stem of Gymnosperms* (e.g., conifers) is similar to that of many woody dicotyledons in that the activity of a cambium forms a cylinder of secondary phloem centrifugally and a cylinder of secondary xylem centripetally. A transverse section of a *young pine stem* shows a number of interesting anatomical features, viz.:

(*a*) The large cavities or *resin canals* which appear in the cortex and in the secondary xylem (in the latter region the canals are smaller than those in the cortex). Notice that the resin canal (which has arisen by the pulling apart of groups of cells in certain regions and is hence of the *schizogenous* type) is bordered by a ring of small, densely protoplasmic *secretory cells* which exude resinous material into the canal. In certain species of pine, the resin is of great commercial importance and is obtained by "taping" the trees; the resin obtained in this way is the basis of turpentine and allied products.

(*b*) The *secondary xylem* is relatively simple in structure and consists of tracheids, fiber tracheids and uniseriate xylem rays. A

study of the secondary phloem has already been made in Exercise XI.

4. *The stem of monocotyledons.* The stem in many members of this group is characterized by the fact that the stele consists of *collateral bundles* which are more or less scattered through the axis, i.e., they are not arranged in the form of a single "ring" as in dicotyledons. As a consequence, the limits of cortex, pericycle and pith are usually impossible to determine with exactness. However, the recent work of Stover (1934) clearly shows that the disposition of vascular bundles in the stems of grasses does not conform to a single type. Indeed, some genera (*Leerzia, Agropyron*) possess a single series of bundles arranged in a cylinder between the cortex and pith. In most monocotyledonous stems, cambial activity is vestigial or usually absent from the vascular bundles which are thus wholly primary in structure. Such bundles are often termed "closed" bundles in contrast to the "open" bundles of dicotyledons which possess secondary growth.

Obtain a transverse section of a *corn stem* and note under low power the characteristic arrangement of the *collateral vascular bundles* which are more numerous near the periphery of the stem than in the center. Study one of the large bundles under *high power*, noting that the *phloem* (composed of sieve-tubes and companion cells) is directed towards the periphery of the stem while the *xylem* is nearest the center of the stem. The xylem consists of four large primary xylem vessels (which are arranged like the eyes, nose and mouth of a face) and a number of smaller tracheae; a prominent *air-lacuna* is usually present at the inner edge of the innermost large vessel. The bundle is more or less completely surrounded by fibers, a feature very commonly found in the bundles of monocotyledonous stems.

5. *The vine type of stem in dicotyledons.* This type of stem often has its vascular system in the form of a "ring" of bundles which are separated from each other by broad medullary rays composed of parenchyma cells. These radially directed sheets of parenchyma cells may extend vertically the length of an internode or more and in many cases continue to grow (by means of an interfascicular cambium) at the same rate as the fascicular cambium which is increasing the size of the vascular bundles.

Obtain a transverse section of a one-year-old stem of the "Dutchman's Pipe" (*Aristolochia*) and examine it under low power. The following tissues and regions can be seen in progressing from the edge of the section to its center, viz.:

(*a*) A typical *uniseriate epidermis* composed of rather densely protoplasmic cells. Note the extremely thick *cuticle* which covers the epidermis.

(*b*) Internal to the epidermis occurs the *cortex*, formed of an outer zone of rather thin-walled "angular" *collenchyma* cells and an inner zone of large "isodiametric" *parenchyma* cells; note the presence of large *druses* in many of the parenchyma cells of the cortex.

(*c*) The *stele* is sharply delimited by a broad cylinder of closely joined *pericyclic fibers*, the secondary walls of which are still increasing in thickness. Internal to the pericyclic fibers occurs the *parenchyma* of the pericycle which is quite similar in general structure to the cortical parenchyma.

(*d*) The *vascular system* of the *stele* consists of a ring of typical *collateral bundles* in each of which the clearly distinct phloem and xylem is separated by a cambial zone (the *fascicular cambium*). Notice that an *interfascicular cambium* has arisen as the result of the tangential division of certain of the parenchyma cells of the broad *medullary rays* which separate the bundles.

The *pith* of the stem is large and is composed of "isodiametric" parenchyma cells in many of which druses are evident.

Next obtain a transverse section of a two- or three-year-old stem and notice the *profound changes* in structure which have been occasioned by *secondary growth*, viz.:

(*a*) A *discontinuous* and rather thick layer of *phellem* (cork) has appeared as the result of the activity of the phellogen layer. Notice particularly how the epidermis has been forcibly broken away from the cortex; strips of epidermal tissue are visible on the outer surface of the corky tissue. A comparatively extensive development of *phelloderm* can also be seen internal to the phellogen. *Lenticels* are well-developed at certain points.

(*b*) The previously *continuous* cylinder of *collenchyma* has been broken as the result of the pressure of secondary growth.

Notice that parenchyma cells of the cortex have intruded into the gaps between the strips of collenchyma cells.

(*c*) The *previously continuous* cylinder of *pericyclic fibers* has likewise been ruptured and the gaps filled up by the parenchyma cells from the cortex. Notice the beginnings of wall-thickness and lignification in some of the parenchyma cells between the strips of fibers; these parenchyma cells will finally become thick-walled *stone-cells* (by a process of secondary sclerosis) and will thus effectively "repair" the broken mechanical cylinder. (For further details of this phenomenon, cf. the Introduction of Exercise VIII.)

(*d*) The *medullary rays* between the vascular bundles have become broad and long and their component parenchyma cells are arranged in more or less definite *radial rows* as the result of the continued activity of the *interfascicular cambium*.

(*e*) Each *vascular bundle* has increased enormously in size as the result of the continued activity of the *fascicular cambium*. Notice the *crushed condition* of the cells in the outer portion of the *phloem:* this has resulted from the centrifugal development of additional secondary phloem. The *xylem portion* of each vascular bundle shows three definite *annual rings;* notice that the largest vessels occur at the edge (i.e., in the "spring wood") of each annual ring.

(*f*) The irregularly-shaped *pith* is now much reduced in extent and shows clear indications of crushing. *Aristolochia* represents one of the exceptional cases where the pith is actually compressed as the result of secondary growth.

III. Suggested Drawings and Notes.—

1. Prepare a large diagrammatic drawing of the trans-section of the stem of *Pelargonium* indicating by legends or by labels the position and relative extent of all the tissues and regions.

2. Prepare a drawing similar to the above of the trans-section of the stem of *Tilia*.

3. Draw a lenticel (of *Sambucus*, or *Aristolochia*) as seen in median longi-sectional view showing carefully the complementary tissue and the closing layers and the relation of these tissues to the adjacent periderm of the stem. Label all important structures.

4. Prepare enlarged drawings of the periderm of the stem of *Pelargonium*, *Tilia* or *Sambucus* showing the arrangement and structure of the cells of the phellem, phellogen and phelloderm. Examine macerated bottle-cork (i.e. the phellem of the cork oak, *Quercus suber*) and sketch several of the individual cells.

5. Draw in detail the resin canals as seen in trans-sections of the stem of *Pinus*.

6. Show, by a diagram, the arrangement of the vascular bundles as seen in the trans-section of the corn stem. Draw in detail a single vascular bundle showing the fibrous bundle-sheath and the structure of the primary phloem and primary xylem.

7. Prepare diagrams of the trans-sections of the stem of *Aristolochia*. These diagrams should illustrate the effects of secondary growth on the primary structure of the stem.

8. Examine the demonstrations of various types of nodal anatomy and prepare diagrams to illustrate them.

9. Outline the processes of mitosis and cytokinesis as they occur in the fusiform initials of the cambium in gymnosperms (cf. Bailey 1920 and 1923).

10. Outline the methods of secondary growth in the stem of woody monocotyledons (cf. Chamberlain 1921 and Cheadle 1937).

REFERENCES

1. Arber, A., The Interpretation of Leaf and Root in the Angiosperms. Biol. Rev. 16:81-105. 1941.
2. Bailey, I. W., The Cambium and Its Derivative Tissues, III. A reconnaissance of cytologieal phenomena in the cambium. Amer. Jour. Bot. 7:417-434. 1920.
3. ————————, ——, The cambium and Its Derivative Tissues, IV. The increase in girth of the cambium. *Ibid.* 10:499-509. 1923.
4. Barthelmess, A. Ueber den Zusammenhang zwischen Blattstellung und Stelenbau unter besonderer Berüchsichtigung der Koniferen. Bot. Archiv. 37:207-260. 1935.
5. Boke, N. H., Histogenesis and Morphology of the Phyllode in Certain Species of *Acacia*. Amer. Jour. Bot. 27:73-90. 1940.
6. Boke, N. H. Zonation in the shoot apices of *Trichocereus spachianus* and *Opuntia cylindrica*. Amer. Jour. Bot. 28:656-664. 1941.

7. Cheadle, V. I., Secondary Growth by Means of a Thickening Ring in Certain Monocotyledons. Bot. Gaz. 98:535-555. 1937.
8. Chamberlain, C. J., Growth Rings in a Monocotyl. Bot. Gaz. 72:293-304. 1921.
9. De Bary, Ch. XIV, XV.
10. Eames, A. J., The Vascular Anatomy of the Flower with Refutation of the Theory of Carpel Polymorphism. Amer. Jour. Bot. 18:147-188. 1931.
11. Eames and MacDaniels, Chs. V, VI, IX, XI.
12. Esau, K., Hilgardia, Ontogeny and Structure of the Phloem of Tobacco. *Ibid.*, 11:343-424. 1938.
13. ————, ——, Phloem Anatomy of Tobacco Affected with Curly Top and Mosaic. Hilgardia 13:437-490. 1941.
14. Foster, A. S., Problems of Structure, Growth and Evolution in the Shoot Apex of Seed Plants. Bot. Rev. 5:454-470. 1939.
15. Grégoire, V., La morphogénèse et l'autonomie morphologique de l'appareil floral. La Cellule 47:287-452. 1938.
16. Hayward, Ch. III.
17. Helm, J., Untersuchungen über die Differenzierung der Sprossscheitelmeristeme von Dicotylen unter besonderer Berücksichtigung des Procambiums. Planta 15:105-191. 1932.
18. Jeffrey, Ch. XIII.
19. Kaplan, R., Ueber die Bildung der Stele aus dem Urmeristem von Pteridophyten und Spermatophyten. Planta 27:224-268. 1937.
20. Küster, E., Sekundäres Dickenwachstum; Holz und Rinde. Allgemeine Einletung. Handbuch d. Pflanzenanatomie. IX. Berlin. 1939.
21. Louis, J., L'ontogénèse du système conducteur dans la pousse feuillée des Dicotylées et des Gymnospermes. La Cellule 44: 87-172. 1935.
22. Smith, G. M., Cryptogamic Botany. Vol. II. Ch. V. New York. 1938.
23. Sperlich, A., Das trophische Parenchym. B. Excretionsgewebe. Handb. d. Pflanzenanatomie. IV. Berlin, 1939.
24. Stover, E. L., Development and Differentiation of Tissues in the Stem Tips of Grasses. Ohio Jour. Sci. 34:150-160. 1934.
25. Wilson, C. L. and Just, T., The Morphology of the Flower. Bot. Rev. 5:97-131. 1939.

Exercise XIII

THE LEAF

I. Introduction.—As stated in the previous exercise, it is difficult, on both theoretical as well as practical grounds, to demarcate rigidly the leaf from the stem. If, as several morphologists have suggested, the "leaves" of higher plants arose phylogenetically from determinate branch systems, this difficulty at once becomes understandable. Indeed, perhaps the most useful character which distinguishes the leaf from the stem, apart from its origin at the shoot apex, is the *early cessation of apical growth*. The leaves of ferns retain an apical meristem for a relatively long period in their development, but in seed plants the final size and form of the leaf is largely determined by *intercalary growth*. Leaves are without much question the most diversified of all the "organs" produced by higher tracheophytes (cf. Troll 1938-1939, and Arber 1941). The foliage leaf, which is the most familiar type, varies from the small scale-like structures found in certain gymnosperms and angiosperms to the enormous and complex leaves of palms. In addition to foliage leaves, other types of foliar organs must be considered under the morphological concept of "leaf." As illustrations may be mentioned cotyledons, bud scales, bracts, and according to classical theory, the appendages of the flower. In view of such morphological and functional diversity, it is obviously impossible to generalize with respect to the histological structure of "leaves." From an anatomical standpoint, the leaf may be regarded as an "expansion" of the axis in which all the fundamental primary tissue regions (i.e., epidermis, cortex and stele) may be recognized. But the arrangement and structure of the photosynthetic parenchyma (i.e., the *mesophyll*), the vascular system (i.e., the major and minor veins) and the mechanical tissues (e.g., collenchyma, sclereides and fibers) vary within extremely wide limits.

In this exercise, a brief study will be made of a few representative leaf types, with particular emphasis upon the anatomy of the leaf-blade or lamina. It is beyond the scope of this book to discuss the process of leaf origin and the differentiation of the various leaf tissues in seed plants. For information on these matters, reference should be made to Foster (1936), Hayward (Ch. III, pp. 77-85), Troll (1938), and Cross (1940, 1941).

II. Material for the Study of Leaf Anatomy.—

1. *The lamina of the foliage leaf of lilac* (*Syringa vulgaris*). Obtain a stained trans-section of the lamina and study its histology under low magnification. Note first of all the clear distinction between the *midrib* and the two thin lateral flaps of tissue. An examination of the midrib reveals a large *collateral vascular bundle* in which the phloem is directed towards the *abaxial* or lower leaf-surface while the xylem is situated beneath the *adaxial* or upper leaf surface. With this orientation in mind, it will now be clear that the lamina exhibits a typical *dorsiventral character*, shown not only by the relative positions of xylem and phloem in the larger veins but also by the differentiation of the mesophyll, into *palisade* and *spongy parenchyma*. Since the anatomy of the lamina in the region of the veins differs somewhat from the interveinal areas, it will be more convenient to describe briefly the various tissues and then to point out their topographical variations. In the lamina of this leaf, three principal tissues are present, viz.:

(*a*) *The epidermis.* The *adaxial epidermis* consists of somewhat oval cells, the outer walls of which are covered by a thin cuticle. Although exact measurements are lacking, there seems to be relatively little difference in the thickness of the inner, outer and radial walls of the epidermis. Observe that many of the epidermal cells possess a protoplast which is peripheral in position. *Stomata* are not uncommon in the adaxial epidermis. Note particularly the relatively small size of the guard cells and the air chamber present beneath each stomate. *Trichomes*, represented by capitate and simple hairs, occasionally develop but these structures are more abundant on the abaxial surface of the lamina. The *abaxial epidermis* is fundamentally similar to the adaxial epidermis except that the cells are somewhat smaller and thinner-

walled. Stomata are more abundant in this layer of the lamina, a common situation in many angiosperms. "Stalked" stomata are frequently present in the midrib region. *Multicellular capitate hairs*, lying within shallow depressions, are relatively common and consist of a terminal group of densely protoplasmic *secretory cells* (with rather thick outer walls) which are seated upon a small unicellular stalk. Haberlandt (p. 240) suggests that the capitate hairs of *Syringa* may absorb thin films of water from the leaf surface but this supposed function requires further investigation.

(b) The *mesophyll*. This tissue region is composed of two types of parenchyma, viz.: (1) the *palisade parenchyma* which is found directly beneath the adaxial epidermis and consists of rather narrow, thin-walled, somewhat "rectangular" cells, the long axes of which are perpendicular to the epidermis. Notice that *intercellular air spaces* are prominently developed in the palisade parenchyma. In addition to a prominent nucleus, each palisade parenchyma cell contains a large number of peripheral *chloroplasts* in which the process of photosynthesis is carried on. Directly internal to the abaxial epidermis occurs (2) the *spongy parenchyma* which is composed of thin-walled irregular cells which have no definite orientation and are very loosely arranged. Notice that in many instances adjacent cells of the spongy parenchyma touch each other at their narrowest points so that the maximum of wall surface borders upon the large *air spaces*. The cells contain a peripheral protoplast and a smaller number of chloroplasts than occurs in the cells of the palisade parenchyma. The spongy parenchyma has at least two important *functions*. First, because of its loosely arranged cells, it acts as a "ventilating tissue" of the leaf, i.e., diffusion of CO_2, water vapor and O_2 between the air lacunae and the cells can take place with relative ease. Second, the spongy parenchyma carries on some photosynthesis although this function is more efficiently performed by the palisade parenchyma. According to Haberlandt (p. 287) no trans-location takes place from one palisade-cell to another but "the stream of synthetic products" follows the *long axes* of these elements. Some anatomical evidence in support of this view is furnished by the fact that small groups of 2-10 palisade cells in

certain plants converge at their lower ends and rest upon the upper dilated end of a cell of the spongy parenchyma. Haberlandt regards these specialized cells of the spongy parenchyma as *collecting cells* which receive the products of photosynthesis from the palisade cells and transmit them directly or indirectly to the main vascular channels.

(*c*) *The vascular system.* Investigate first of all the general structure of the midrib, and note particularly the absence of photosynthetic parenchyma from this portion of the blade. Instead of the usual spongy and palisade parenchyma, the median vascular bundle is covered on both sides by a number of layers of isodiametric thick-walled cells, the outermost of which are thickened in such a manner as to resemble collenchyma. (Note: *This "replacement" of photosynthetic parenchyma by collenchyma and thick-walled parenchyma occurs to a less extent in connection with the smaller vascular bundles of the blade.* In other types of leaves sclerenchyma may be present—mechanically a "girder effect" is produced.) Several smaller vascular bundles with the phloem towards the adaxial surface are usually seen in the upper part of the midrib; these "*accessory bundles*" unite basally with the single leaf trace. A certain amount of *secondary growth* has taken place in the median vascular bundle, a phenomenon which is of rather general occurrence in the larger veins of dicotyledonous leaves. The conspicuous *secondary xylem* is composed of conducting elements (probably both *tracheids* and *vessels*) and *fibers* arranged in more or less definite radial rows and uniseriate *xylem rays* which extend across the "*cambial zone*" into the *phloem.* The *primary xylem* (which lies above the secondary xylem) consists of cells which show more or less of a radial arrangement and which are imbedded among *xylem parenchyma.* The *secondary phloem* consists of polygonal thin-walled *sieve-tubes* which are associated with small somewhat triangular densely protoplasmic *companion cells, phloem parenchyma,* and *uniseriate phloem rays,* each of which frequently terminates in a large parenchyma cell. The *primary phloem* is indefinite and difficult to distinguish. The "*cambial zone*" can be distinguished but is not nearly as prominent or distinct as in the case of a stem. Nevertheless, three or more radial rows of differentiating cells

can be rather clearly seen. In general, cambial activity is never prolonged in the veins of most leaves. In the smaller veins of the lamina, the vascular tissue is considerably reduced in amount and secondary growth may be entirely lacking. In certain regions of the blade, the diverging bundles may be cut more or less obliquely so that the characteristic type of primary xylem elements may be recognized. The very small *leaf veins* may consist of several parenchyma cells and a few primary xylem elements; a bundle of this character is usually surrounded by a jacket of parenchyma cells containing chloroplasts (i.e., the so-called "*border parenchyma*").

2. *The leaf blade of corn* (*Zea Mays*). Obtain a transverse section of a corn leaf and examine it under *low power*. The *adaxial epidermis* is readily identified by the presence of groups (3-5 cells) of somewhat lens-shaped, apparently empty cells. These cells are known as *bulliform cells* and by changes in their turgor allow the leaf-blade to curl or uncurl, a phenomenon which may be advantageous in restricting the loss of water from the leaf under arid conditions. The typical *epidermal cells* of both the abaxial and adaxial epidermis are somewhat oval in transverse section (actually they are rather elongated cells) and are provided with a definite cuticle. *Stomata*, with conspicuous air-chambers beneath them, are present in both epidermal layers. Occasional unicellular, sharp-pointed *hairs* occur on the adaxial epidermis.

The *mesophyll* tissue of the leaf shows no clear differentiation into palisade and spongy parenchyma but instead is composed of several layers of rather compact parenchyma cells.

The *vascular system* of the leaf consists of a parallel series of *collateral bundles*. The majority of the bundles are rather small; at intervals, fairly large bundles occur. Examining one of the *small bundles* under high power, note that it is completely surrounded by a *bundle sheath* of rather large "isodiametric" parenchyma cells which contain chloroplasts; the bundle sheath, sometimes termed the "*mestome sheath*," may act as a conducting layer which presumably transports the products of photosynthesis directly to the vascular system, i.e., the phloem. The *xylem* of each bundle is directed towards the adaxial surface of the leaf and consists only of small tracheae. The *phloem* of the bundle

is nearest the abaxial surface of the leaf and at most is formed of a few small *sieve-tubes* and *companion cells;* in very small bundles, the phloem may be represented by parenchyma cells. The structure of a *large vascular bundle* in the leaf is quite similar to the anatomy of a stem bundle (cf. Exercise XII). Notice particularly the clear distinction between the sieve-tubes and companion cells of the phloem and the presence of an air lacuna at the adaxial edge of the xylem. *Fibers* are present on both edges of the bundle and may even partially surround it; notice the thick-walled character of the epidermal cells directly adjacent to the fibers. This arrangement of fibers on either side of the vascular bundle is quite characteristic of grass leaves and is regarded as a very efficient "plan" for securing mechanical strength in the leaf.

3. *The bud scale.* In general, bud scales are distinguished anatomically from foliage leaves by (1) their greatly reduced vascular system, which may consist of a series of parallel or dichotomizing veins, and (2) by a simple type of undifferentiated mesophyll. The outer bud scales of certain trees may produce a well-developed *periderm* beneath the outer epidermis (e.g., *Aesculus*). Mechanical cells, such as *fibers* and *sclereides*, are often prominent for example in *Camellia*, *Fagus*, *Quercus*, and *Populus*. (For further details, consult Foster, 1928, pp. 137-146.) Study prepared trans-sections of the bud scales of several of the forms listed above.

4. *The leaves of gymnosperms.* Examine stained trans-sections of the needles of *Pinus* noting especially the sunken stomata, the invaginated walls of the mesophyll cells and the vascular bundle (in some species two bundles are present) embedded in *transfusion tissue*. (Cf. Eames and MacDaniels, pp. 307-308.) For comparison, study trans-sections of the fan-shaped lamina of the foliage leaf of *Ginkgo biloba*. (A discussion of the histology of this leaf is given by Chamberlain, 1935, pp. 191-193, and Fig. 210, p. 196.)

III. Suggested Drawings and Notes.—

1. Prepare a diagrammatic drawing of the trans-section of the lilac leaf, indicating the position and extent of all important

tissues. Draw, showing cellular detail, a narrow sector through the thin portion of the blade. This drawing should include a vein. Label all cells and important structures.

2. Draw in detail the cellular structure of a small sector through the corn leaf. This drawing should include a group of bulliform cells and at least one well-developed vein.

3. Prepare diagrams to illustrate the structure and the arrangement of tissues in the bud scale of some angiosperm and in the foliage leaves of *Pinus*.

4. Outline the differentiation of a "typical" dicotyledonous leaf, such as *Nicotiana* or *Pelargonium* (cf. Foster, 1936).

5. Explain what is meant by the expressions "sun leaves" and "shade leaves." (Cf. Haberlandt, pp. 295-297.)

REFERENCES

1. Arber, A., The Interpretation of Leaf and Root in the Angiosperms. Biol. Rev. 16:81-105. 1941.
2. Chamberlain, C. J., Gymnosperms, Structure and Evolution. Chicago. 1935.
3. Cross, G. L. Development of the foliage leaves of *Taxodium distichum*. Amer. Jour. Bot. 27:471-482. 1940.
4. ————. Some histogenetic features of the shoot of *Cryptomeria japonica*. Amer. Jour. Bot. 28:573-582. 1941.
5. Eames and MacDaniels, Ch. XII.
6. Foster, A. S., Salient Features of the Problem of Bud-scale Morphology. Biol. Rev. 3:123-164. 1928.
7. ————, ——. Leaf Differentiation in Angiosperms. Bot. Rev. 2:349-372. 1936.
8. Haberlandt, Ch. VI.
9. Hayward, Ch. III, pp. 75-87.
10. Jeffrey, Ch. XIV.
11. Meyer, F. J., Das trophische Parenchym. A. Assimilationsgewebe. Handb. d. Pflanzenanatomie. IV. Berlin, 1923.
12. Pfeiffer, H. Die pflanzlichen Trennungsgewebe. Handb. d. Pflanzenanatomie. V. 1928.
13. Troll, W., Vergleichende Morphologie der höheren Pflanzen. Bd. I. Zweiter Teil. 1-4. Lieferung. (Morphologie des Blattes) Berlin, 1938-39.

Exercise XIV

THE ROOT

I. Introduction.—Except in the Psilotales, *Salvinia* and a few specialized parasitic forms, roots are a typical feature of the sporophyte of vascular plants. In many of the lower tracheophytes, the primary root is short-lived and numerous adventitious roots soon begin development from various portions of the shoot system. In seed plants, however, the radicle often reaches great prominence and in such cases is termed a *tap root*. Additional *fibrous roots* in seed plants arise by successive branchings beginning with the first root but are also commonly formed adventitiously from the stem. Roots perform a number of important physiological functions. Primarily, they serve as organs which absorb water and solutes from the soil solution. In addition, they are very important as structures which anchor the plant firmly in the soil. The storage of reserve food material also occurs in most roots to some extent and is very obviously displayed in the fleshy "roots" of carrot, beet, turnip and similar economic plants. Although the usual environment of roots is the soil, *aerial roots* are produced in certain vines which serve to attach the shoot firmly to the surface upon which it may be growing.

1. *Origin and structure of the primary tissues of the root.* The primary structure of the root differs from that of the stem in a number of respects. The following brief resumé of these differences will emphasize the salient histological characteristics of the root, viz.:

(*a*) *The structure and growth of the root apex.* In marked contrast to the *superficial position* of the terminal meristem of the shoot, the root apex consists externally of a *root cap* which acts as a protective buffer to the delicate meristem beneath it. A wide variety of "types" of apical structure have been described

for the roots of various seed plants (cf. Hayward, pp. 44-47), but it is evident that this region of the root deserves further intensive study with the aid of modern botanical microtechnique (cf. von Guttenberg, 1940, 1941). The essential point here is that the *activity* of the terminal meristem of the root is fundamentally different from that of the shoot apex. In the latter, exogenous leaf primordia arise from the flanks or base of the apex. But in the root, two dissimilar patterns of differentiation originate from the terminal meristem, one leading to the *outward addition* of new cells to the root cap, the other contributing new cells to the main but *unsegmented* body of the root. These differences are at present impossible to explain but it is evident that they determine the fundamental morphological differences between root and shoot (cf. Arber, 1941).

(*b*) *Primary tissue regions.* Young roots, prior to secondary growth, resemble stems in the presence of epidermal, cortical and stelar regions. The *epidermis* of roots is usually devoid of a *cuticle* and *stomata* and its chief rôle appears to be that of absorption, a process which is favored by the development, behind the region of elongation, of a *zone of root hairs.* The *cortex* of roots is often entirely composed of thin-walled *storage parenchyma* and is soon destroyed if secondary growth occurs. Apparently the *endodermis*, which is commonly regarded as the innermost layer of the cortex, is a consistent feature of roots, in contrast to its variable distribution in the stem. But without doubt one of the most fundamental characteristics of the young root is shown by the arrangement and development of the *primary vascular tissues of the stele.* In striking contrast to the *collateral* position of xylem and phloem in the siphonostele of typical stems, these tissues are arranged in a *radial* and *alternate* pattern in the root. Thus, in the anatomical sense, there are no true primary vascular bundles in the stele of the root. As seen in trans-sectional view, the primary xylem and phloem of the root appear as separate and alternating strands of tissue. Very frequently, the *xylem "plates"* as they are often termed, meet in the center to form a solid core. In many roots, however, particularly in monocotyledons, the center of the stele is occupied by a core of

parenchyma which resembles the *pith* region of the stem. Although the number of phloem and xylem groups in a given root is equal, variations occur between the roots of the same plant as well as between different species (cf. Esau, 1941, p. 452, Table 1). There appears to be no satisfactory explanation for the inconstancy in the number of phloem and xylem strands so characteristic of the roots of some angiosperms. Depending upon the number of primary xylem groups, the stele is described as *diarch* (2), *triarch* (3), *tetrarch* (4), *pentarch* (5), etc. The stele in monocotyledons usually consists of many alternating xylem and phloem groups and hence is designated as *polyarch*.

In further contrast to the stem of seed plants, the primary xylem of the root is *exarch*. This means that the radial maturation of the tracheary elements from provascular tissue occurs in the *centripetal direction*. Hence the *protoxylem* lies at the outermost edge of each xylem strand, next to the *pericycle*, while the *metaxylem* is situated towards the center of the stele. Since exarch primary xylem is only found in the stems of the lower vascular plants (e.g., the Psilopsida and Lycopsida), the root of seed plants has been regarded by some anatomists as a "conservative" or "primitive" organ (cf. Jeffrey, Ch. XII). Much work needs to be done on the comparative histogenesis of the primary tissues in the root. In several recent studies, Esau (1940, 1941) has made important contributions to our knowledge in this direction. It is interesting to note that her observations indicate that the phloem begins to differentiate nearer the apical meristem than does the xylem. In the *protophloem* of carrot, for example, "the sieve-tubes complete their differentiation about 300 microns from the apex of the root" whereas the first protoxylem elements "show secondary walls approximately 1 millimeter from the root apex, but do not lose their protoplasts through another millimeter of the root."

(*c*) *Origin of lateral roots.* Branching of the stem normally originates from lateral buds which develop at or near the shoot apex from superficial cells or cell layers. In marked contrast, the *branching of the root is strictly endogenous*. The origin of lateral root primordia usually occurs through the renewed growth and division of certain cells in the *pericycle* of the stele distal to

the zone of root hairs. According to Hayward (p. 51), in some plants the adjacent cells of the endodermis may contribute to the formation of the lateral root primordium. Arnold (1940) has shown that in the water hyacinth (*Eichhornia crassipes*) lateral roots arise in the "immature pericycle" and "at the forward end of the region of elongation." Because of its internal origin, the further development of the lateral root involves its penetration through the endodermis, cortex and epidermis of the mother root to the outside. Just how this occurs is not entirely clear. The suggestion has been made that the mechanical pressure exerted by the emerging lateral root may also be accompanied by some kind of chemical dissolution of the tissues interposed in its path. In roots with three or more xylem plates, lateral root primordia typically appear opposite each of the protoxylem points. Consequently, unless injuries or abnormalities occur, the lateral roots tend to emerge in vertical rows which are equal in number to the xylem groups. But in diarch steles, lateral roots may appear at each side of the two phloem groups. In this case there would be four vertical rows of lateral roots (cf. Esau, 1940, pp. 190-194).

2. *Secondary growth in roots.* The roots of many herbaceous dicotyledons and of all woody plants exhibit secondary growth in thickness. However, because of the radial, alternate arrangement of the primary vascular tissues, the *cambium* first appears as separate bands of periclinally-dividing cells which originate from parenchyma cells internal to each phloem group. At these points formation of *secondary* phloem outwardly, and *secondly xylem* inwardly, occurs as in a typical stem. In *woody* plants, the originally separate strips of cambium finally become united laterally as a result of tangential divisions in the pericycle external to each xylem group. Thus at an early stage, the vascular cambium in this type appears lobed in trans-section. Ultimately, by the formation of secondary phloem and xylem external to the xylem plates, the contour of the cambium becomes cylindrical. In roots of this type, the *primary xylem* eventually becomes completely surrounded by a cylinder of secondary xylem. In certain *herbaceous plants*, in contrast, the cambium, at points opposite the protoxylem points, forms broad *parenchymatous rays* so that a

dissected type of secondary vascular cylinder results (Jeffrey, pp. 156-157 and Hayward, pp. 48-51). When secondary growth is pronounced in a root, the primary phloem, cortex and epidermis soon become crushed and slough away. In trees, a typical "bark" is produced and, save for the exarch primary xylem in the center, all structural resemblance with a root is lost. The first *periderm* layer of the root arises by the formation of a phellogen in the pericycle. Later-formed phellogen layers may subsequently appear, as in the stem, from living cells in the secondary phloem.

II. Material for the Study of the Root.

1. *The root of buttercup* (*Ranunculus* sp.). Obtain a stained trans-section of the root and study the following tissues and regions beginning at the edge of the section:

(*a*) The *epidermis*, a uniseriate but broken layer of collapsed and partially destroyed cells. The imperfect condition of the epidermis presumably is due to the abrasive effect of the soil on the root. Notice that a more or less disorganized protoplast is visible in some of the epidermal cells.

(*b*) Within the epidermis occurs the rather broad, homogeneous *cortex* which is composed entirely of rather thin-walled, "isodiametric" parenchyma cells most of which are separated from one another by prominent intercellular air spaces. Observe that while the outer layers of the cortex are composed of rather tightly joined empty cells (forming a "hypodermis"), the inner cortical cells contain prominent starch grains. Large, somewhat irregular *simple pits* are visible on the end walls of the cortical parenchyma cells.

(*c*) The center of the root is occupied by the *stele* which is externally separated from the cortex by a uniseriate cylinder of cells, the *endodermis*. Study the endodermis under high power, noting the presence of a protoplast in many of the cells. The salient feature of the endodermis (in the primary condition) is the presence of a suberized or cutinized band which extends completely about the inner surface of the radial and end walls of each cell. These band-like thickenings of the wall are known as *Casparian strips* and in recent years have received

a great deal of attention because of the apparent physiological importance of the endodermis as a cellular layer which regulates the entry of water and mineral salts into the vascular system of the stele. The assumption is made that the suberized nature of the Casparian strip renders it impermeable to water so that diffusion must take place through the tangential walls and the protoplasts of the endodermal cells, i.e., through a semi-permeable membrane. However, the exact function and significance of the endodermis is in need of much further investigation.

In the endodermis of *Ranunculus*, the presence of the Casparian strip is indicated by the *red color* of the short radial walls. Individual cells of the endodermis may have uniformly and heavily thickened walls, a phenomenon previously recorded by Caspary in the case of *Ranunculus Ficaria* (cf. De Bary, p. 123). In the roots of certain plants, all of the cells of the endodermis may be thick-walled in character (cf. Eames and MacDaniels, p. 102, Fig. 51). Within the endodermis occurs a single layer of living cells which is known as the *pericycle*. The most conspicuous portion of the stele is represented by the *primary vascular tissue* which shows the characteristic *radial arrangement* of the xylem and phloem goups. It will be seen that four radial plates of xylem (which join in the center of the root, thus forming a protostele) are present; the stele is therefore designated as *tetrarch*. The *protoxylem* (which presumably consists of annular and spiral elements) is represented by several very small "polygonal," thick-walled cells found at the outer edge of each xylem plate. The *metaxylem* (which probably is composed of pitted elements) consists of much larger cells and as stated previously forms a homogeneous tissue in the center of the root; a pith is thus absent. Laterally adjacent to each xylem plate near its outer edge, you will find one or two rather thick-walled cells, which are approximately hexagonal as seen in transverse section and which appear distinct from the xylem because of their lighter-staining walls and the possession of a more or less disintegrated protoplast. The shape and structure of these cells suggest that they are sieve-tubes; their exact morphology is difficult to interpret but they seem to belong to the phloem. Each of the four *phloem groups* is separated laterally and internally from

the xylem by one or more layers of parenchyma. The phloem consists of thin-walled living cells which, as in many plants, are distinguished with difficulty from the surrounding parenchyma.

2. The root of *Smilax*, *Zea* or some other monocotyledonous type. Examine carefully the trans-section, noting particularly the *polyarch* stele and the central pith-like region.

3. *The origin of lateral roots.* Study transverse and longi-sections of the root of the water hyacinth (*Eichhornia crassipes*) and observe the method of origin and early ontogeny of the lateral root primordia. For comparative purposes, make a similar study of lateral root development in bean or willow.

4. *Secondary growth in roots.* Study a series of trans-sections of the root of a woody or herbaceous plant cut at levels successively distal to the region of maturation of the primary vascular system. Observe the method of origin of the cambium and the formation of secondary vascular tissues and the periderm.

III. Suggested Drawings and Notes.—

1. Prepare diagrammatic drawings of trans-sections of the root of *Ranunculus* and of some monocotyledonous type showing the position and extent of all the primary tissues. *Draw in detail* a portion of the stele in each type to illustrate the structure of the primary phloem and xylem.

2. Prepare diagrams based upon both transverse and longi-sections to illustrate the origin of lateral roots and their emergence to the surface of the mother root.

3. Prepare a series of diagrams based upon material studied in the laboratory to illustrate the origin of the cambium and the effects of secondary growth upon the primary tissues of the root.

REFERENCES

1. Arber, A., The Interpretation of Leaf and Root in the Angiosperms. Biol. Rev. 16:81-105. 1941.
2. Arnold, C. A., A Note on the Origin of the Lateral Rootlets of *Eichhornia crassipes* (Mart.) Solms. Amer. Jour. Bot. 27:728-730. 1940.
3. De Bary, pp. 348-366.
4. Eames and MacDaniels, Ch. X.

5. Esau, K., Developmental Anatomy of the Fleshy Storage Organ of *Daucus carota*. Hilgardia 13:175-226. 1940.
6. ————, ——, Phloem Anatomy of Tobacco Affected with Curly Top and Mosaic. *Ibid.*, 13:437-490. 1941.
7. Hayward, Ch. H.
8. Jeffrey, Ch. XII.
9. Von Guttenberg, H. Der primäre Bau der Angiospermenwurzel. Handb. d. Pflanzenanatomie. VIII. Berlin, 1940.
10. ————, Der primäre Bau der Gymnospermenwurzel. *Ibid.*: Berlin, 1941.

APPENDIX

The following brief notes on certain phases of microtechnique are given here to facilitate the use of this book by the teacher and student. For full information on the various procedures used in preparing tissue for microscopic study, reference should be made to the publications of Chamberlain (1932), Rawlins (1933), Johansen (1940), and Sass (1940), cited under "General References."

Free-hand Sections

In many of the exercises in this book, directions are given for the study of sections cut by hand from living stems, leaves or other plant structures. To prepare such material requires only simple technique and in addition provides a realistic picture of cells and tissues which should precede the examination of microtomed and permanently-stained preparations. In the laboratory the student can acquire the necessary skill with a sectioning-razor to enable him to explore the structure of such tissues as the epidermis, parenchyma and collenchyma. Sections cut by hand should be carefully mounted on a clean slide either in distilled water or in the various reagents designated and the cover-glass lowered gently into place. For more resistant cells, such as sclereides or fibers and for the critical study of the sieve-plates in phloem elements, the use of the carbon dioxide freezing microtome is highly desirable. With the aid of this instrument, a large number of thin sections may be prepared by the instructor in advance of class use. The student must learn to check free-hand preparations at frequent intervals so that the sections are not allowed to dry out. Cells immersed in fluid are not only easier to study from an optical point of view but they also retain a more or less normal structure over a relatively long period of observation. Sections of hairy objects, such as many leaves or stems, are often difficult to mount in water without the formation of numerous air-bubbles. This difficulty may be removed by

mounting such sections in a weak solution of alcohol. This may act as a killing reagent for the protoplasm but it does make possible the accurate study of the shape, arrangement and character of the walls of cells.

Prepared Slides

The use of permanent slides is essential in the study of many of the topics outlined in this book. This is particularly true for the work to be done under Exercises III, X, XII, XIII, and XIV. Suitable preparations as a basis for class study are obtainable from commercial supply houses or may be prepared for the student directly. With reference to the latter possibility, detailed suggestions for the collection, fixation, sectioning, and staining of tissues and organs are presented systematically in the recent manuals on microtechnique by Johansen (1940) and Sass (1940).

Macerated Tissue

One of the most important skills which the student must develop in laboratory practice is the ability to visualize cells as three-dimensional bodies. This is often extremely difficult on the basis of the examination of sections which tend to create a two-dimensional concept. Furthermore, many definitive features of cells, particularly the structure and arrangement of pits and fibrous thickenings in tracheary elements, and the character of perforations in vessel elements, can best be studied in isolated cells. For these reasons, a study of macerated tissue is recommended for many topics in this book and is especially desirable in connection with Exercises II, VIII, IX, and X. The maceration of plant tissue is most effectively accomplished by the use of certain reagents which dissolve the intercellular substance and thus cause the separation of a piece of tissue into its component cells. Jeffrey's method is usually satisfactory. Small pieces of the material, no thicker than a match, are placed in a glass vial containing a mixture of equal parts of 10% chromic acid and 10% nitric acid. The vial is then corked and placed in an electric oven at a temperature of 30°-40° C. until the material becomes soft or "mushy" in texture. Hard material, such as

wood and the shells of nuts may require several days in the oven, during which time it is advisable to change the macerating fluid once or twice. Boiling small slivers of wood before placing them in the acids drives out the air and accelerates the maceration process. The macerated tissue is carefully washed in distilled water to remove as much of the acid as possible and can then be transferred to 50% alcohol for future study. Often effective results may be secured by staining the isolated cells in safranin. For class use, it is only necessary to agitate the alcoholic suspension of cells and to add a drop with a pipette to a slide in order to secure a fairly representative "sample" of the desired cell types. Permanent preparations of macerated tissue are easily made by placing small quantities of cells in water on a slide, evaporating the excess water on an electric hot-plate and mounting in glycerine-jelly. Circular cover-glasses should be used, the edges of which can be sealed with some type of cement which prevents drying out and the entrance of air.

Special Reagents

1. Phloroglucin and Hydrochloric Acid.—The addition of these reagents produces a red color in the walls of sclereides, lignified fibers and tracheary elements (cf. Exercises VIII, IX, and X). The stain is not permanent but nevertheless is extremely useful in demarcating the thick walls of certain types of cells. A saturated solution of phloroglucinol should be prepared in 95% alcohol. Mount the section or tissue fragment directly in a drop of this reagent on the slide and add a cover slip. Then introduce a drop of concentrated hydrochloric acid at the edge of the cover slip. Great care should be taken to carry out this procedure some distance away from the microscope.

2. Potassium Iodide (IKI) and Sulphuric Acid.—This is a specific test for cellulose. Mount the sections in the potassium iodide (1 gr. iodine and 3 g. potassium iodide in 300 cc. of distilled water, according to Rawlins, 1933) and add a cover slip. The introduction of a drop of 65% sulphuric acid will cause cellulose walls to turn blue in color.

3. Aniline Blue.—This is a specific stain for callus depositions on sieve-plates and is essential for the procedure outlined in Exercise XI. Sections should be immersed for a short time in a .1% aqueous solution, and then transferred, after gentle washing, to a drop of water. The callus on the sieve-plates is stained blue. Dr. A. S. Crafts has suggested to the writer the following improvement: Place the sections in IKI, wash in water, stain in aniline blue for about five minutes, then wash briefly again with IKI and mount for study in tap water or glycerine.

4. Neutral Red.—This vital stain is very useful in staining the vacuome of plant cells and is recommended for use in Exercises V, VI, and VII. Mount the sections directly in a .1% aqueous solution.

5. Sudan IV.—This reagent is specific for the cuticle, and for cutinized and suberized cell walls. Place the sections in a drop of alcoholic solution of Sudan IV (.5 g. in 100 cc. of 80% alcohol) on a slide and warm gently over an alcohol flame. Add a cover-glass and examine under the microscope. The cuticle, as well as waxy materials present in walls are stained red. This reagent is very desirable for use with Exercise V.

INDEX